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RESEARCH ARTICLE

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Large-scale drivers of relationships between soil microbial properties and organic carbon across Europe

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Abstract

Aim: Quantify direct and indirect relationships between soil microbial community properties (potential basal respiration, microbial biomass) and abiotic factors (soil, climate) in three major land-cover types.

Location: Europe.

Time period: 2018.

Major taxa studied: Microbial community (fungi and bacteria).

Methods: We collected 881 soil samples from across Europe in the framework of the Land Use/Land Cover Area Frame Survey (LUCAS). We measured potential soil basal respiration at 20 °C and microbial biomass (substrate-induced respiration) using an O_2-microcompensation apparatus. Soil and climate data were obtained from the same LUCAS survey and online databases. Structural equation models (SEMs) were used to quantify relationships between variables, and equations extracted from SEMs were used to create predictive maps. Fatty acid methyl esters were measured in a subset of samples to distinguish fungal from bacterial biomass.

Results: Soil microbial properties in croplands were more heavily affected by climate variables than those in forests. Potential soil basal respiration and microbial biomass were correlated in forests but decoupled in grasslands and croplands, where microbial biomass depended on soil carbon. Forests had a higher ratio of fungi to bacteria than grasslands or croplands.

Main conclusions: Soil microbial communities in grasslands and croplands are likely carbon-limited in comparison with those in forests, and forests have a higher dominance of fungi indicating differences in microbial community composition. Notably, the often already-degraded soils of croplands could be more vulnerable to climate change than more natural soils. The provided maps show potentially vulnerable areas that should be explicitly accounted for in future management plans to protect soil carbon and slow the increasing vulnerability of European soils to climate change.

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KEYWORDS

climate change, croplands, Europe, land cover, soil carbon, soil microbial biomass, soil microbial respiration, structural equation modelling

1 | INTRODUCTION

Soils harbour *c*. 25% of global biodiversity and are critical for multiple ecosystem functions and services (Geisen et al., 2019; Wall et al., 2012), contributing to large-scale processes such as climate regulation (van den Hoogen et al., 2019; Wieder et al., 2013) and key biogeochemical cycles (Crowther et al., 2019). As soils store more carbon than the atmosphere and aboveground plant biomass combined, global carbon dynamics are particularly dependent on soils (Xu et al., 2013). Specifically, soil organic carbon stocks are related to local primary productivity, litter decomposition rates, and soil microbial activity (Cagnarini et al., 2019; Gleixner, 2013; Malik et al., 2018). These factors, together with other soil (e.g., structure, texture, and water content) and climatic (e.g., temperature and precipitation) variables, shape soil carbon dynamics. Despite the importance of microbial properties (e.g., activity, biomass) for soil carbon, our understanding of these contributions is still limited, and current carbon modelling approaches often do not consider soil microbial activity (Wieder et al., 2013; Yigini & Panagos, 2016), particularly at large spatial scales.

One holistic measure of soil microbial activity is microbial respiration, the process by which available soil carbon is respired into CO_2 to create energy and further microbial products that contribute to long-term soil carbon storage (Crowther et al., 2019; Schmidt et al., 2011). The level of respiration is related to the abundance (often represented as microbial biomass; Serna-Chavez et al., 2013) and community composition of soil microorganisms, which in turn are affected by the quantity and quality of organic carbon substrates available to support microbial activity (Eisenhauer et al., 2010; Tiunov & Scheu, 2004). Another common metric of soil microbial community functioning is the respiratory quotient, defined as the ratio of microbial respiration to microbial biomass. It has been interpreted variably as a measure of microbial efficiency (Anderson & Domsch, 1985) and an indicator of ecosystem stress (Wardle & Ghani, 1995). By this interpretation, a lower respiratory quotient corresponds to a more efficient or less stressed community, as the given amount of microbial biomass can be supported with a lower basal respiration. Microbial activity also varies depending on the composition of the microbial community. For example, fungi-dominated communities are generally associated with slower organic matter turnover, causing an expectation of lower basal respiration compared to bacterial-dominated communities (Crowther et al., 2019).

Overall, the activity, biomass, efficiency and composition of soil microbial communities are co-determined by many environmental factors, whose relative importance varies across different land-cover types, biogeographical regions and studies (Hendershot et al., 2017; Serna-Chavez et al., 2013; Xu et al., 2017). For instance, while a study of 84 forest, grassland and shrubland sites in North America (Colman & Schimel, 2013) found that pH did not have much of an effect on microbial respiration, suggesting that the known effect of pH was subsumed into climate effects, an 81-site pan-European survey of forests, grasslands, and arable lands (Creamer et al., 2016) reported that climate, as defined by biogeographical zone, did not affect basal or multiple substrate-induced respiration, whereas pH was an important explanatory factor. Moreover, a meta-analysis of approximately 1,000 soil communities from all continents except Australia across a range of land-use intensity levels did not find any consistent pattern in microbial abundance (or diversity) measures across elevational or latitudinal gradients (Hendershot et al., 2017). Taken together, these inconsistent findings show that more large-scale studies, using standardized methodologies to determine the drivers of soil microbial properties, are needed not only to assess the significance of specific drivers, but also to infer their potential context-dependencies.

The response of soil microbial communities to the drivers listed above can be shaped in part by land-cover type (Goss-Souza et al., 2017). For example, the microbial response to warming can depend on the amount of substrate (i.e., litter/plant biomass input) available (Xue et al., 2016), which is very different among (and even within) different land-cover types. Furthermore, the response of soil microbial variables, such as respiration and biomass to substrate availability, is affected by soil moisture and climate, and this interaction can differ in forests and arable lands (Geisseler et al., 2011; Liu et al., 2009; Sun et al., 2020; Wang et al., 2016). Such context-dependencies prompt individual analyses and explicit comparisons of different land-cover types to arrive at a more comprehensive understanding of how diverse microbial communities respond to abiotic drivers under diverse conditions.

More comprehensive insights into the determinants of, and interactions between, soil carbon, soil microbial activity, biomass, and carbon-use efficiency would allow better understanding of the fine balance between soil carbon release and storage (Classen et al., 2015). As microbial processes are incontrovertibly a crucial part of carbon cycling (Schmidt et al., 2011), including them in modelling of soil carbon gives a more complete picture of processes, such as soil carbon turnover and stabilization, sharpening the accuracy of predictions for more effective land management and restoration strategies and to provide better estimations of the soil carbon pool in the future (Cagnarini et al., 2019; Crowther et al., 2019; Schmidt et al., 2011; Wieder et al., 2013).

Here, we provide a high-resolution cross-climate dataset on soil microbial biomass and respiration from soils with different land-cover types (i.e., forest, grassland, cropland) to assess the relationship between soil microbial communities and soil carbon. We measured soil

microbial potential basal respiration, biomass, and respiratory quotient in soil samples from 881 sites distributed across the European Union (Orgiazzi et al., 2018). To our knowledge, no previous study has addressed these important soil microbial properties in such a standardized way at this macroecological scale using a single consistent method in a single laboratory (in contrast to meta-analyses). We used this large-scale and spatially comprehensive dataset to investigate two hypotheses: (a) climate, soil, and geographical characteristics affect soil microbial respiration and biomass in different ways across land-cover types, and (b) these differences also manifest in how microbial properties (here microbial biomass, potential basal respiration) relate to soil carbon. The obtained results also allow the generation of predictive maps of soil respiration and microbial biomass at a continental scale, which will be of fundamental interest to further interrogate the future impacts of climate change, land use and soil restoration.

2 | METHODS

Soil samples were collected from 881 unique sites covering most European environmental conditions under the auspices of the Land Use/Land Cover Area Frame Survey (LUCAS) from April to December 2018, to a depth of 20 cm (Orgiazzi et al., 2018). They were stored on ice and transported to Ispra, Italy. From there, they were transported to Leipzig, Germany in March 2019, for the measurement of potential basal respiration by O_2-microcompensation (Scheu, 1992), microbial biomass by substrate-induced respiration (Anderson & Domsch, 1978), respiratory quotient (qO2; the ratio of basal respiration to microbial biomass), and gravimetric water content. A random subset of 267 samples was transported from Ispra to the Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas (CEBAS-CSIC, Murcia, Spain) for the measurement of ester-linked fatty acid methyl esters (FAMEs) as indicators of bacterial and fungal biomass (Vera et al., 2021). As all samples were measured at 20 °C, which for most samples did not correspond to field temperature conditions, basal respiration and qO_2 represent potential rather than actual microbial activity (Xu et al., 2017). We therefore also used an equation based on the temperature sensitivity of basal respiration to estimate actual qO_2, as in Xu et al. (2017). As basal respiration is highly dependent upon soil water content, water holding capacity was measured in February 2021 for a subset of 101 samples (at least 30 from each land-cover type), followed by two remeasurements of potential basal respiration: one at field capacity, and one after adjusting each sample to 60% water holding capacity. Soil organic carbon and other soil properties, measured with International Organization for Standardization (ISO) methods, were taken from the LUCAS 2018 survey (Orgiazzi et al., 2018); climate data were obtained from online databases (Supporting Information Appendix S1). For a full description of methods, see Supporting Information Appendix S2.

Statistical analyses were performed in R version 3.6.1 (R Core Team, 2019). Potential basal respiration, microbial biomass, qO_2, and soil organic carbon content were all log 10 transformed prior to analysis. Since potential basal respiration and qO_2 values include zeros, 0.001 was added to each prior to log transformation. The lowest non-zero values were on the order of 0.1 for basal respiration and 0.0001 for qO_2. Potential basal respiration, microbial biomass, and qO_2 values for one sample were removed due to a measurement error. All variables were standardized by subtracting the mean and dividing by the standard deviation prior to the following analysis to enable meaningful comparison of effect sizes. An ANOVA and Tukey honestly significant difference (HSD) test was performed on each variable to assess trends across land-cover types.

Structural equation models (SEMs) were constructed using lavaan version 0.6.5 (Rosseel, 2012; R Core Team, 2019). The hypothesized SEM structure and justifications are available in Supporting Information Table S2-2. We included samples from 185 grasslands (self-seeded or sown permanent communities of grasses, grass-like herbs and forbs, including pastures), 289 forests (areas with at least 10% tree canopy), 347 croplands (anywhere crops are cultivated) and 64 samples from other land-cover types including shrublands, bare land, and urban areas (9 samples were removed due to unavailable climate data). The purpose of this general model was to unravel drivers of soil microbial respiration and biomass without any regard to land cover; so, we included all available samples from the full range of land-cover types of the LUCAS Soil Biodiversity survey. Although these 59 samples from other land-cover types were used to parameterize the general SEM, they were not used when looking at the effects of the individual land-cover types as replication was not representative at the European scale.

To investigate whether and how different land-cover types affect soil properties in different ways, we also used multigroup analysis to consider the effects of three broad land-cover types (forest, cropland and grassland) individually, creating an SEM for each land-cover type (excluding the 'other' category), to explore potential differences in the relationships between soil, climate, geographical and microbial parameters. This enables more precise predictions of soil microbial properties and carbon, as models are parameterized to the conditions of the different land-cover types. For in-depth definitions of the different land-cover categories, see Supporting Information Table S3-3 and E4.LUCAS (ESTAT) (2018).

We tested the justification of our grouping by comparing a model where all paths were constrained across land-cover types to the model allowed to freely vary, using a chi-squared difference test to assess statistically significant differences between the two (statistical difference implies justification). We followed the same procedure to test whether specific land-cover types had significantly different models and to test for significant differences in individual paths between the land-cover types. For a more detailed explanation of this testing procedure, as well as the full justification of the underlying metamodel SEM, see Supporting Information Appendix S2 and Table S2-2.

We used the underlying structural equations of the SEMs to create predictive maps of potential basal respiration and microbial biomass across the European Union with a monthly step for 2018 and

then aggregated to obtain an average for the year. This was done because predictions are specific to a 30-day time period, due to inclusion of the mean temperature and total precipitation in the 30 days before sampling. These maps show broad, large-scale geographical patterns – they are not intended for numerical prediction of microbial properties at any one given site. Predictions were made for the three respective land-cover types and then aggregated to a single spatial representation across Europe. We included the same climate and geographical data used in the original model. Sand content, pH, and carbon maps came from studies published by the European Soil Data Center (ESDAC), based on LUCAS 2009/2012 soil property data (Ballabio et al., 2016, 2019; Panagos et al., 2012; Yigini & Panagos, 2016). The predictive maps do not include any urban areas or areas above 1,000 m a.s.l., as these were not included in the modelled organic carbon map we used as input and were not (well-) represented in the LUCAS data used to create the models (Yigini & Panagos, 2016).

To validate our maps, we first compared the predictions of the obtained maps to our measured values. To do so, we extracted the predicted potential basal respiration and microbial biomass from the monthly map corresponding to the month in which each sample had been taken and then calculated the correlation between these predicted values and the actual measured values. We used the land-cover specific models to make these maps, so they only included areas classified as cropland, grassland or forest by the LUCAS survey. Six hundred and thirty-six of our initial sample sites were in areas covered by the maps and could be used for this cross-validation. We followed the same procedure for the predictions created from the annually aggregated maps, both with and without considering land-cover type. As a final validation step, we also tested the correlations between our predicted values and the observations from an independent dataset of 269 samples from individual plots across 145 sites in Europe from the International Soil Biogeography Consortium (iSBio; Heintz-Buschart et al., 2020). For these samples, the same methods to determine both potential basal respiration and microbial biomass were used, and all samples came from natural ecosystems or control plots of the iSBio experiments to ensure that they correspond to non-manipulated conditions at each location. Of 145 sites covered by our general predictive maps, 125 fell into one of our three land-cover types of interest and could be used to validate the land-cover-specific maps. Of these 125 sites, 65 were forests, 34 were croplands and 26 were grasslands.

We additionally estimated the environmental coverage of the current sampling design to evaluate the spatial uncertainty of our predictions. For this, we used the Mahalanobis distance, which estimates a multidimensional distance, and defined outliers as the 97.5% quantile of the chi-squared distribution with *n* degrees of freedom (Jackson & Chen, 2004; Rousseeuw & van Zomeren, 1990); in our case 6 corresponding to annual precipitation and mean temperature, soil pH, soil carbon, sand content, and elevation. This algorithm allowed us to identify regions where our predictive maps are more or less reliable (Supporting Information Figure S3-6; Jackson & Chen, 2004; Rousseeuw & van Zomeren, 1990).

3 | RESULTS

Forest sites generally received more precipitation than croplands, both in the month preceding sampling as well as annually (Supporting Information Figure S3-3). Cropland sites were generally at lower elevations than forests and grasslands, and tended to be warmer and drier as shown by ANOVAs and Tukey HSD tests. Additionally, most forests were at higher latitude sites, and had higher sand content than the other two land-cover types (i.e., grasslands and croplands). Grasslands also had higher sand content than croplands. Water content, pH, soil carbon, and potential basal respiration were significantly different across all three land-cover types. Forests had the highest potential basal respiration, qO_2, soil organic carbon, and water content, and the lowest pH; grasslands displayed intermediate values for all of these properties. Croplands had the lowest potential basal respiration, qO_2, soil organic carbon, and water content, and the highest pH. Cropland sites had the lowest microbial biomass; however, forests and grasslands did not differ from one another. To further validate our results, we performed a temperature adjustment following the same methodology as Xu et al. (2017), in order to shift from potential to actual microbial activity. While this approach consistently lowered the qO_2 values of the samples, it did not change the patterns among land-cover types (see Supporting Information Appendix S5).

The samples measured without adjustment of water content showed significant differences in potential basal respiration between forest and the other land-cover types (ANOVA/Tukey HSD: forest–grassland $p = .018$, forest–cropland $p < .01$, grassland–cropland $p = .18$; Supporting Information Table S3-4). This remained the case when the eight forest samples with a field water content over 30% were removed from the analysis. When the samples were adjusted to 60% water holding capacity, there were no significant differences between land-cover types (ANOVA; $p = .462$; Supporting Information Table S3-4).

3.1 | Structural equation modelling

The general SEM, which did not consider land-cover type, showed significant relationships between potential basal respiration, soil organic carbon, and microbial biomass (Figure 1a; C_{mic}-BAS: covariance (cov) = 0.119, $p = .002$; C_{mic}-SOC: cov = 0.448, $p < .001$, BAS-SOC: path coefficient = 0.144, $p < .001$, model fit estimates: chi-squared of 0.239, $p = .888$). Climate as a whole (i.e., monthly and annual precipitation and temperature) had a strong effect on all response variables, with direct positive effects of higher mean annual precipitation on potential basal respiration (0.143, $p < .001$), microbial biomass (0.218, $p < .000$) and soil organic carbon (0.234, $p < .001$). Higher mean annual temperature was directly related to higher soil carbon, but indirectly decreased soil organic carbon (direct = 0.446, $p < .001$; indirect = −0.202, $p < .001$; Figure 1). Higher mean annual temperature also related to lower potential basal respiration through direct effects (−0.480, $p < .001$; Figure 1). Temperature and precipitation in the month

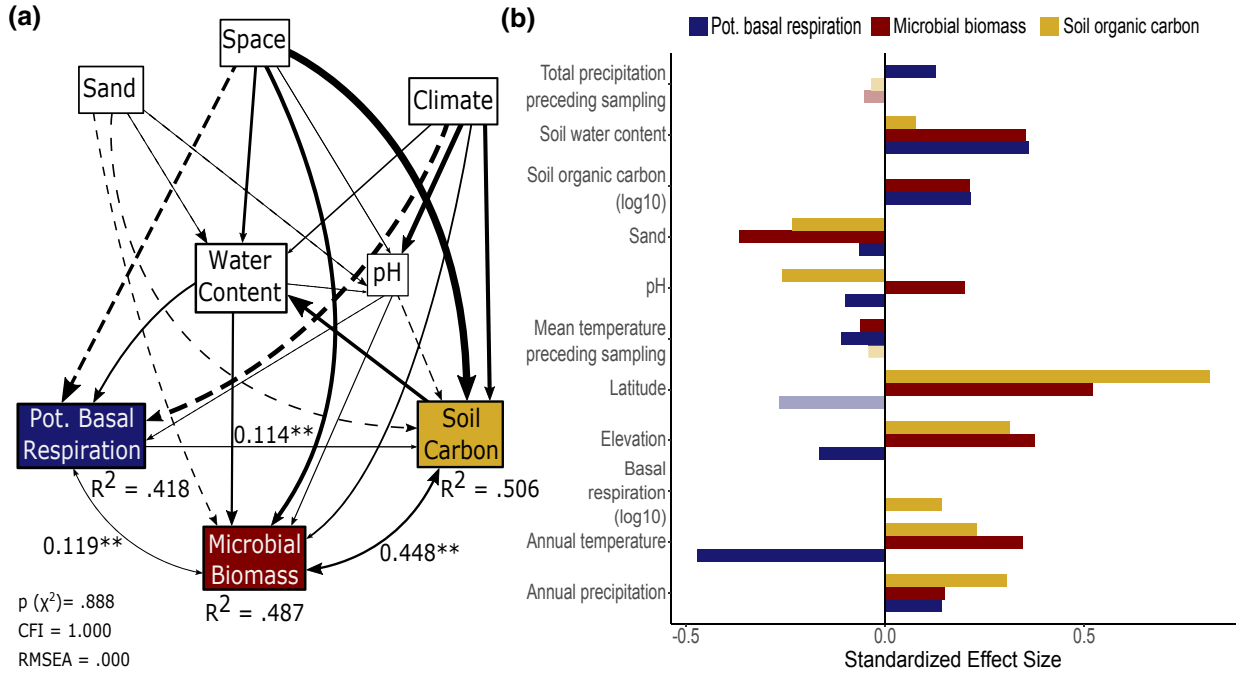

FIGURE 1 The results of the general structural equation model (SEM) created using data from 872 sampling points across Europe (without differentiating between land-cover type). (a) Path diagram showing SEM results. Line thickness corresponds to strength of relationship based on standardized path coefficients. Dashed lines indicate a negative relationship; solid lines are positive. Only significant relationships are shown. The path coefficient and covariances between potential basal respiration, soil organic carbon, and microbial biomass are also shown numerically; here, a double asterisk (**) indicates that the path is significant ($p < 0.05$). The climate variable encompasses measures of temperature and precipitation, each with a monthly and yearly value; space represents elevation and latitude. Double-headed arrows indicate a correlation rather than a causal relationship due to potential reciprocal effects; numbers and line thickness for them represent the covariance. CFI stands for comparative fit index and RMSEA for root mean square error of approximation, both measures of SEM fit. (b) The total (sum of direct and indirect) effect sizes of each relationship. Transparency indicates that the total effect was not significant ($p > .05$)

prior to sampling had significant total effects on potential basal respiration (temp: total = −0.110, $p < .001$; precip: total = 0.127, $p < .001$), but not on microbial biomass or on soil carbon, except for a very slight negative total effect of temperature prior to sampling on microbial biomass (−0.061, $p = .040$). High latitude and elevation were directly associated with higher soil organic carbon and microbial biomass (Figure 1), representing the main effects shaping soil carbon at this scale. High latitude additionally had a strong and significant indirect effect on microbial biomass (0.142, $p = .007$). Elevation and latitude directly significantly decreased potential basal respiration (elev: −0.191, $p = .010$; lat: −0.390, $p = .004$). For full numerical quantification of all relationships, see Supporting Information Appendix S6, Table S6-9.

The overall multigroup analysis model had a chi-squared of 4.967 and a p-value of .761, with eight degrees of freedom. The non-constrained model had an AIC of 7,960 and was thus significantly better than the constrained model, which had an AIC of 8,568 (chi-squared difference test, $p < .001$), indicating our grouping was justified. The paths affecting potential basal respiration and soil carbon were not significantly different between forests and grasslands ($p = .294$); paths affecting pH did not differ significantly between forests and croplands ($p = .670$); paths affecting soil carbon were not significantly different between grasslands and croplands ($p = .120$; Supporting Information Table S3-5). Other than these exceptions, all paths were significantly different between all land-cover types.

In the grouped models, some differences among the land-cover types were observed. Climate, driven in large part by annual temperature, affected C_{mic} most strongly in croplands (Figure 2, Supporting Information Table S6-8). In forests, however, none of the climate variables had a significant effect on soil microbial biomass. In grasslands, the annual precipitation significantly increased soil microbial biomass (annual precip. = 0.320, $p < .001$). Additionally, the correlation between potential basal respiration and microbial biomass was found to be strong and positive in forests (.455, $p < .001$), weaker and non-significant in grasslands (.103, $p = .355$), and slightly negative but non-significant in cropland areas (−.055, $p = .336$). The opposite trend was observed for the correlation between soil carbon and microbial biomass, which were less strongly correlated in forests (.235, $p = .003$) than in grasslands and cropland areas (.490, $p < .001$; .396, $p < .001$, respectively). Soil carbon increased with latitude and elevation across land-cover types; however, this effect was most pronounced in forests, and there was no significant effect of elevation in croplands or grasslands. This contrasts with the relatively strong positive effects of both latitude and elevation on microbial biomass in croplands, which were less pronounced in forests and not present in grasslands.

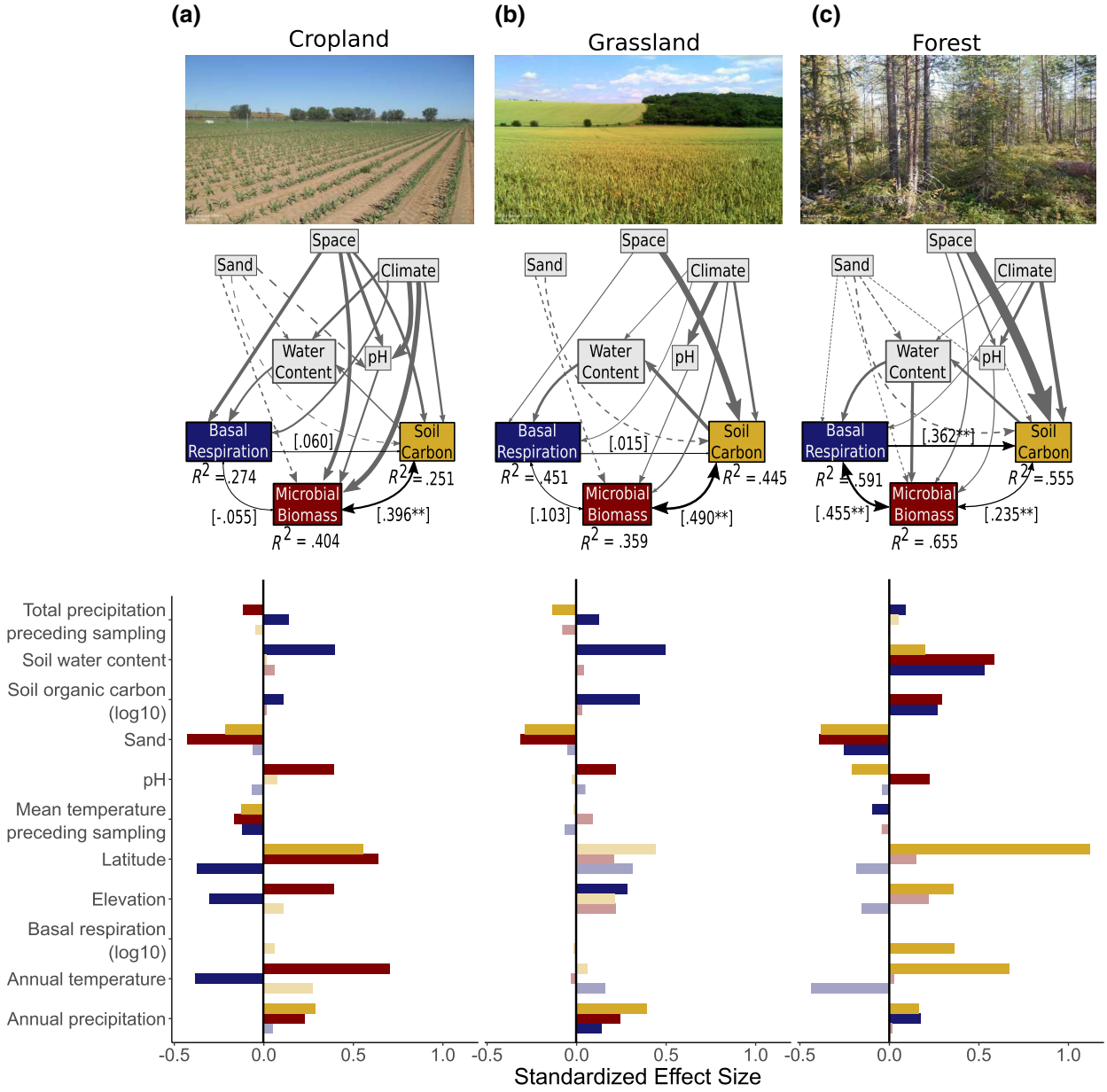

FIGURE 2 The results of the multigroup structural equation model (SEM) analysis, which results in a separate SEM for each land-cover group. Model comparative fit index (CFI) = 1.000, root mean square error of approximation (RMSEA) = .000, *p*-value (chi-squared) = .761. In the path diagrams, only significant paths for each group are shown, except for the relationships between potential basal respiration, soil carbon, and microbial biomass (black lines), which are shown regardless of significance. For these, a double asterisk (**) indicates that the path is significant ($p < 0.05$). Line thickness corresponds to the size of path coefficients. Dashed lines indicate a negative relationship; solid lines are positive. Double-headed arrows indicate a correlation rather than a directed relationship due to potential reciprocal effects; numbers and line thickness for them represent the covariance. R^2 for each predicted quantity is the amount of variance in that variable explained by the model. The climate variable encompasses measures of temperature and precipitation, each with a monthly and yearly value; space represents elevation and latitude. In the bar graphs, transparency indicates that the total effect was not significant ($p > .05$). Bars show the standardized total effect size (sum of direct and indirect effects). (a) croplands; (b) grasslands; (c) forest. Images from the Land Use/Land Cover Area Frame Survey (LUCAS) 2015 photo viewer (https://ec.europa.eu/eurostat/web/lucas/lucas-photo-viewer)

3.2 | Predictive mapping

Predictive mapping averaged over 2018 (with land-cover type accounted for) showed that forested Scandinavia had high values of both microbial biomass and potential basal respiration (Figure 3b & c). Microbial biomass was very variable throughout Europe, without any clear latitudinal trends. Potential basal respiration generally had mid-range values throughout the middle of Europe, with higher values in alpine areas

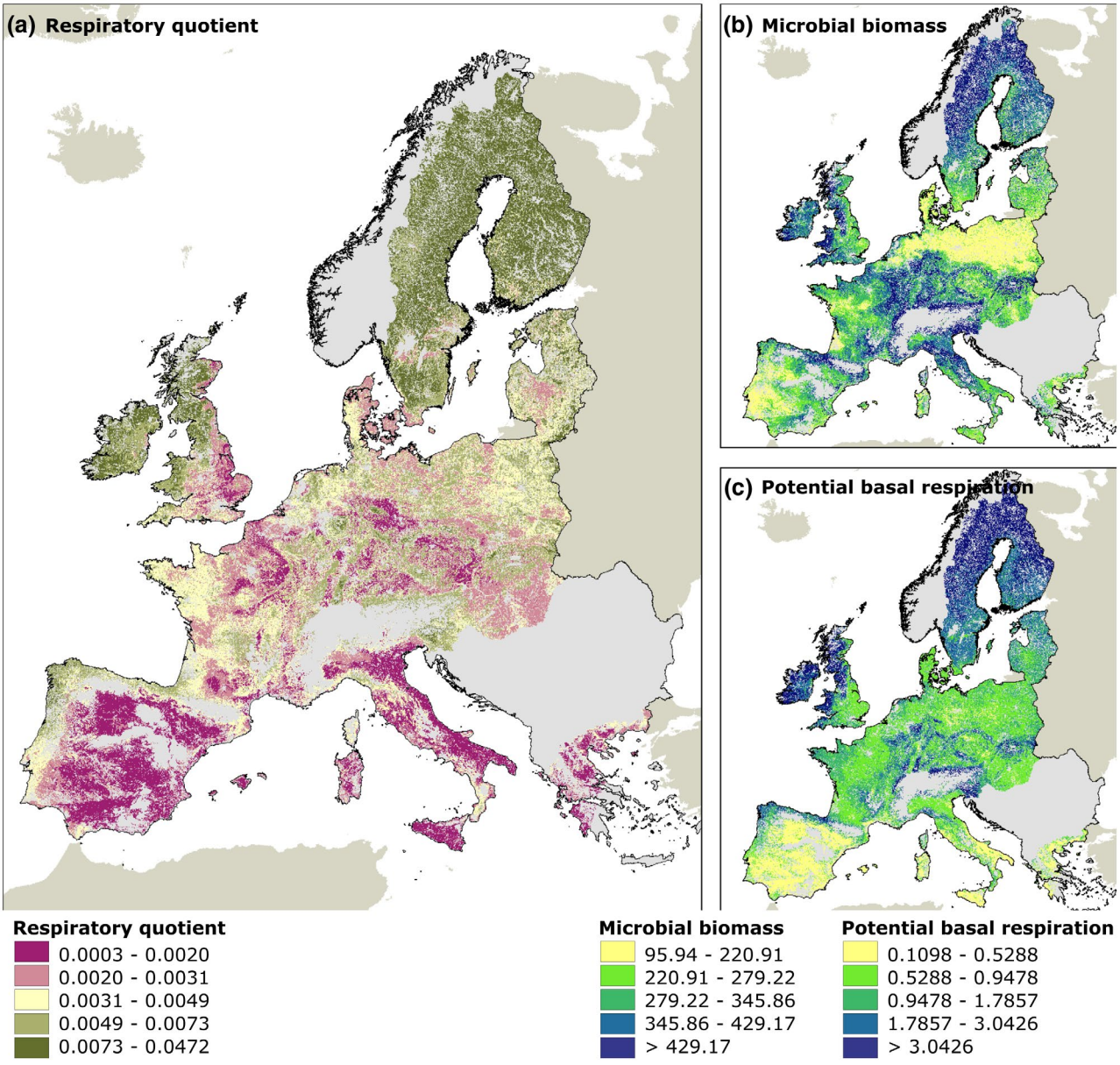

FIGURE 3 Predictive maps of mean microbial biomass, potential basal respiration at 20 °C, and respiratory quotient (qO_2) in 2018 across the European Union, excluding altitudes over 1,000 m, created by averaging the predictive maps created for each month of 2018. (a) Modelled qO2 (μL O2/(μg Cmic·hr)) across Europe; (b) Mean microbial biomass (μg C_{mic}/g soil dry weight); (c) mean potential basal respiration (μL O_2/(g soil dry weight·hr)). Maps of standard deviation and Mahalanobis distance error estimation are available in Supporting Information Appendix S3, Figures S3-4, S3-5 and S3-6

and lower values towards the south of Spain. Potential basal respiration showed the highest annual variation in Spain and Italy, whereas microbial biomass showed more moderate variation in these areas, with highest variation occurring in Eastern Europe (Supporting Information Figures S3-4, S3-5).

The predicted values for the corresponding month extracted from the maps were significantly positively correlated with the actual observed microbial biomass and potential basal respiration data (C_{mic}: Spearman's rho = .39, $p < .001$; BAS: Spearman's rho = .62, $p < .001$). These correlations were similar to those calculated using the annual averaged maps with regard to land-cover type (C_{mic}: Spearman's rho = .36, $p < .001$; BAS: Spearman's rho = .61, $p < .001$), and using the general model without considering land-cover type (C_{mic}: Spearman's rho = .37, $p < .001$; BAS: Spearman's rho = .63, $p < .001$).

Data extracted from our predictive maps were also correlated with independent potential basal respiration and microbial biomass data from the iSBio (Heintz-Buschart et al., 2020) dataset. The annual general map had the highest correlation with the iSBio data (C_{mic}: Spearman's rho = .52, $p < .001$; BAS: Spearman's rho = .53, $p < .001$). The annual maps with land cover had the next-best correlation (C_{mic}: Spearman's rho = .40, $p < .001$; BAS: Spearman's rho = .51, $p < .001$), followed by the monthly map with land cover (C_{mic}: Spearman's rho = .40, $p < .001$; BAS: Spearman's rho = .50, $p < .001$).

3.3 | Microbial community structure

Ester-linked fatty acid methyl esters (FAMEs) showed a higher proportion of fungi in forests than in grasslands or croplands, and a higher proportion in croplands than in grasslands (Supporting Information Figure S3-3). Grassland had significantly higher absolute bacterial biomass than the other two land-cover types, and forests had a significantly higher absolute fungal biomass than the other two (Supporting Information Figure S3-3). Neither potential basal respiration nor respiratory quotient had a significant relationship with either fungal or bacterial biomass or fungal : bacterial ratio in any land-cover type (Supporting Information Figures S3-9, S3-10). Microbial biomass was significantly correlated with the total microbial biomass calculated from FAMEs (Supporting Information Figure S3-11; $t = 10.877$, $df = 264$, $p < 2.2e\text{-}16$, Pearson's correlation coefficient = .57).

Temperature adjustment significantly reduced qO_2 values in comparison with the measured values (Supporting Information Appendix S5). However, the patterns in qO_2 were unchanged – that is, forests still showed the highest qO_2, followed by grasslands, followed by croplands (Supporting Information Appendix S5).

4 | DISCUSSION

Soil carbon storage is a dynamic process rather than a matter of stable, discrete pools, and the inclusion of microbial properties is essential in order to be able to understand this process and to model soil carbon dynamics with accuracy (Cagnarini et al., 2019; Gleixner, 2013). Thus, soil microbial properties are not only important for soil science but also have general importance for macroecological understanding of the global carbon cycle (Geisen et al., 2019; Malik et al., 2018). The present study shows that soils under different land-cover regimes are subject to different environmental drivers, and that the way in which their microbial communities interact with soil carbon differs. This finding has potential implications for carbon cycle modelling and land management.

4.1 | Caveats due to laboratory standardizations

We used a standardized approach for taking and measuring samples. Soil samples were taken to a depth of 20 cm and homogenized, which especially in cropland soils likely resulted in some mixing of soil horizons. While this mixing has the potential to influence measurements of potential basal respiration and qO_2, sampling to a standard depth is also necessary to compare such a wide range of soils. Additionally, differences in soil horizons are likely much more pronounced between land-cover types than within (Fang & Moncrieff, 2005), and our land-cover specific models and maps only compare within any one land-cover type, minimizing this effect.

Our other major standardization was measuring all samples at 20 °C, although the soils originated from a large variety of native climate conditions. Unfortunately, it is not feasible to measure each soil at the temperature it would experience in the field. This means that the potential basal respiration we measured does not reflect the actual basal respiration occurring at sites that probably were colder or warmer than 20 °C at the time of sampling, but rather the potential basal respiration that would occur were these soils under these conditions. This may be one reason for the apparent negative relationship between temperature and potential basal respiration; soils from cold areas store large amounts of organic material that is not decomposed in part due to cold temperatures limiting microbial activity. When these soils are brought to 20 °C, the microbial activity can increase accordingly and take advantage of a larger stock of organic material than is stored in soils from naturally warmer areas. As explained above, adjusting values based on the mean annual temperature of the site they were taken from did not change the pattern of our results (Supporting Information Appendix S5). As the substrate-induced respiration measurement of microbial biomass focuses on stimulating as much of the microbial community as possible by optimizing conditions, and the calculation for estimating the microbial biomass based on substrate-induced respiration is standardized to the laboratory temperature conditions, this caveat is less relevant to the substrate-induced respiration measurement (Anderson & Domsch, 1978). It is possible that this is not the single most optimal temperature for every single soil, as some may be adapted to their natural temperature, but measuring at a standardized temperature is both necessary for the sake of comparison and common practice (Anderson & Domsch, 1978; Xu et al., 2013).

Substrate-induced respiration is a soil microbial enzyme-based quantification of microbial biomass, rather than a direct measurement (Anderson & Domsch, 1978). For a reliable measurement, it assumes that a sufficient amount of glucose was provided to saturate the metabolic enzymes of the full microbial community (Anderson & Domsch, 1978). We showed that the 4 mg glucose/g soil dry weight we added was indeed sufficient, as adding more glucose did not influence the estimation of soil microbial biomass (Supporting Information Appendix S4). Glucose is metabolized by the vast majority of microbes; additionally, substrate-induced respiration has been shown to be very well correlated with direct measurements of microbial biomass (e.g., quantified by microscopy/counting; Beck et al., 1997; Lin & Brookes, 1999). Finally, using substrate-induced respiration to quantify microbial biomass carbon is a common technique, as demonstrated by its inclusion in a global meta-analysis of microbial biomass carbon (Xu et al., 2013).

We also chose to perform measurements at field moisture rather than adjusting soils to optimal water-holding capacity. We instead included soil water content in our statistical models, allowing us to visualize the varying magnitudes of the effect that soil water content has across a variety of different soils. Water content can vary greatly at a single site depending on weather leading up to sampling. However, water content is also heavily influenced by local soil properties, such as texture and organic carbon content. Cropland sites have lower soil organic carbon, resulting in a lower water-holding capacity and thus lower water content, regardless of weather. Our

ANOVAs showed that cropland soils consistently had lower water content than forest or grassland soils. Croplands were also consistently in places with lower precipitation in Europe. This pattern cannot be purely a result of any potential bias in sampling date, as forests and cropland sites in particular have a similar distribution of sampling effort across time (Supporting Information Figure S2-2). Additionally, the sampling was designed to represent the distribution of land-cover types across Europe; the predisposition of cropland sites to have lower water content and precipitation is therefore not an artefact of the sampling design, but rather a reflection of the fact that cropland areas in Europe tend to be in places with lower precipitation than forested areas. This pattern in water content is therefore representative of actual conditions and provides insight into the mechanisms behind patterns in potential basal respiration in European soils.

The ability to include soil water content in our models, rather than standardizing it in measurement and being forced to leave it out of models, is also valuable because it is highly variable across different soils and to statistically ignore it would decrease our maps' interpretability and application potential to the real world. If all samples had been measured at the same water content, its direct effect on potential basal respiration would no longer have been visible in the measurement. Our analysis of water holding capacity showed that soil water content is a primary mechanism driving differences in potential basal respiration between land-cover types.

4.2 | Microbial respiration and biomass

The ways in which soil microbial communities, soil properties, and environmental variables interact are complex, particularly at the large spatial scale studied here (Hendershot et al., 2017). The difference can already be seen in the amount of variation explained by our three grouped models – our models did not include information on land management or agricultural practices (e.g., irrigation, fertilization, pesticide use, etc.), which is likely why the model was able to explain considerably less variation in croplands than in grasslands or forests. Additionally, all three categories encompass a broad range of specific land-cover types and management practices, and site legacy, which is not investigated here, can also influence soil organic carbon (Brogniez et al., 2015; de Vries et al., 2012). These caveats notwithstanding, our group models still illuminate crucial differences in microbial processes between the three land-cover types.

The most striking difference is the decoupling of potential basal respiration and microbial biomass in grassland and croplands. In these land-cover types, soil microbial biomass is shown as being significantly correlated with soil organic carbon, but not with potential basal respiration, whereas in forests soil microbial biomass and potential basal respiration were strongly positively correlated. This may indicate general differences in soil microbial community composition (Crowther et al., 2019) as well as carbon limitation of soil microorganisms in grassland and especially cropland soils (Eisenhauer et al., 2010). This potential carbon limitation was not observed in forest soils, which, despite their higher soil potential basal respiration, had higher overall carbon content and showed a positive correlation between soil microbial biomass and potential basal respiration. In forests, the indirect effect of soil organic carbon on potential basal respiration was higher than in croplands or grasslands, but this may be driven by the stronger effect of soil organic carbon on water content and is not indicative of a direct limitation of soil organic carbon on potential basal respiration. In croplands, the water content is likely more dependent upon the management/irrigation scheme than the soil organic carbon, which is why this relationship was not as pronounced in our model. The carbon limitation of soil microbial biomass, as indicated by a correlation between the two variables, is likely driven in part by the lack of a significant organic layer in cropland soils. Forests, on the other hand, do have this organic layer, alleviating the carbon limitation on the microbial community's activity. Although this means that we compared organic and mineral soils, the samples taken still reflect the actual conditions under the different land-cover types; in forests, there is more of an organic horizon, resulting in lower carbon limitation.

One hypothesis for this difference is that forests store more carbon, relaxing carbon limitation associated with soil microbial communities, allowing a greater proportion of the microbial community to actively respire rather than lying dormant until sufficient resources are available. The higher proportion of slow-respiring fungi in forest soils likely also contributes to this relative lack of limitation (Supporting Information Figure S3-3; Crowther et al., 2019). In contrast, bacteria-dominated cropland soils showed no clear relationship between potential basal respiration and microbial biomass, probably due to carbon limitation in the soil (Brogniez et al., 2015; Lal, 2002; Strickland et al., 2019). Nitrogen content and C : N ratio, though not included in this study, could also play a role in this limitation; grasslands and croplands tend to have higher nitrogen availability, which exacerbates carbon limitation (Booth et al., 2005; Eisenhauer et al., 2010; Xu et al., 2013). This situation further limits the microbial community in its functioning potential.

4.3 | Respiratory quotient

The pattern in qO_2 was the opposite of what we expected, being highest in forests and lowest in croplands (Supporting Information Figure S3-3). This is in direct contradiction with the global meta-analysis by Xu et al. (2017), which found that the metabolic quotient was highest in stressed agricultural environments, indicating an inefficient microbial community (Anderson & Domsch, 1978). Although differences between land-cover types were weaker after adjusting for site temperature as done in Xu et al. (2017), indicating that the difference between site and measurement temperature influenced the observed qO_2 pattern, the overall patterns remained (Supporting Information Appendix S5). Another possible mechanism is pH differences, as the pH of forest sites was lower on average than that of cropland or grassland (Supporting Information Figure S3-3), which can lead to a more stressed microbial community and higher qO_2

(Wardle & Ghani, 2018). However, pH was also lower on average in forest sites in Xu et al. (2017); so, this cannot be the whole explanation. We therefore posit that in this context, qO_2 calculated from potential basal respiration and substrate-induced respiration-measured microbial biomass may not only reflect community stress or efficiency, but also resource availability. Basal respiration was more variable than microbial biomass between land-cover types; thus a higher qO_2 indicates that a higher proportion of the microbial biomass measured by substrate-induced respiration was active. Therefore, resource availability in forests must be sufficiently high to allow a relatively large proportion of the microbial community to respire given favourable temperatures. In cropland communities under carbon limitation, a smaller proportion of the microbial community is able to be active. In other words, croplands have less microbial biomass, of which a lower proportion can be active. This explanation is supported by the decoupling between potential basal respiration and microbial biomass in crop- and grasslands explicated above. Enhanced soil microbial respiration may thus reflect microbial processing of organic substrates contributing to soil carbon storage, rather than carbon loss from the soil (Lange et al., 2015).

4.4 | Microbial community composition

Microbial community composition is also crucial to explaining patterns in microbial function (de Vries et al., 2013). Local microbial communities reflect local climate and land-cover conditions as well as agricultural practices, with more disturbed or intensely managed ecosystems tending to have a lower ratio of fungi to bacteria (de Vries et al., 2013; Six et al., 2006; Wubs et al., 2016). Indeed, fatty acid methyl esters (Schutter & Dick, 2000) analysed in a subset of the LUCAS soil samples showed that forest sites had higher fungal biomass than grasslands or croplands, as well as a higher ratio of fungi to bacteria. Grasslands, not croplands, had the lowest ratio of fungi to bacteria (F : B ratio); however, this can likely be attributed to the higher absolute bacterial biomass present in grasslands compared to croplands (Supporting Information Figure S3-3). Fungi are typically slower decomposers of more recalcitrant organic substrates than bacteria, which may be faster decomposers of more labile substrates, and are thus typically associated with higher carbon storage at high latitudes (Crowther et al., 2019). This is supported by the high soil carbon content and fungal biomass observed in our forest samples, of which 45% are from Scandinavia. Within the fungi, slow-cycling ectomycorrhizal fungi are more associated with temperate and boreal forests than arbuscular mycorrhizal fungi, which have faster rates of nutrient turnover (Crowther et al., 2019). This is another compositional factor that could lead to the higher microbial biomass and soil carbon storage, and the corresponding relative lack of carbon limitation, observed in our forest sites. It is worth noting that the sieving procedure may be detrimental to fungi by destroying fungal hyphae, which could lead to an underestimation of total microbial biomass, particularly in fungi-dominated forest systems. However, fungal biomass as measured by fatty acids was correlated with the microbial biomass as measured by substrate-induced respiration, indicating that this technique to quantify microbial biomass does capture fungal biomass as well (Supporting Information Figure S3-11).

4.5 | Predictive mapping and implications

This large-scale survey has allowed us to develop predictive maps of microbial biomass and respiration in Europe. These maps are fundamental to further develop soil condition maps and predict future consequences of climate and land-use changes on soil-based ecosystem services. Soil quality is a complex matter that requires not only the integration of physical and chemical edaphic properties, but also microbial indicators that are more sensitive and respond rapidly to changes in soil conditions (Bastida et al., 2008; Cluzeau et al., 2012; Creamer et al., 2016; Muscolo et al., 2014; Ponge et al., 2013). Such indicators can be particularly useful to assess the response of soil organic matter to these changes, especially if repeated measurements can be considered in the future, which would add a critical temporal component to this analysis (Muscolo et al., 2014). The maps we created are for the purpose of observing broad geographical patterns, and are not meant to provide absolute numerical predictions of soil microbial properties at a given location or territorial unit in Europe. Based on our validation approach, the general model without regard for land-cover type was most effective at predicting soil microbial properties. This is likely due to the large-scale climatic controls observed in our data and the lower fitness of our model for croplands for which other management variables (not considered here) can probably provide more explanatory power (e.g., land-use intensity; Siebert et al., 2019). This once again highlights the importance of considering land-cover type as well as local land-use practices in large-scale modelling in order to be able to obtain the most suitable model parameterization for the environment being modelled (Britz et al., 2011; Pinto-Correia et al., 2016).

Another important result of the grouped models is that microbial biomass and respiration in cropland soils were much more strongly affected by climate variables than those in forests. Soil microbial communities in cropland soils may be more exposed to climatic fluctuations than those in forests, since there is very little vegetation cover, especially after crops have been harvested (though some communities may be adapted to moisture oscillations due to irrigation). This is in line with the findings of a recent study showing that forests with closed canopies are more buffered against the effects of climate change than open-canopy forests (Zellweger et al., 2020). This explanation is supported by the result that in forests, *annual* climate factors, i.e., climate trends, were more influential than *monthly* climate factors, which relate more to immediate weather conditions; in croplands, however, these monthly factors played a larger role. This is in agreement with the overarching finding of Orgiazzi et al. (2016), that cropland soil microbial communities are more at-risk than forest communities across a range of possible threats.

However, the potential effects of climate change on soil microbial communities are not agreed upon (de Vries et al., 2012; Orgiazzi et al., 2016; Rousk et al., 2013). Recent work showed that climate change negatively impacts soil microbial communities across a range of cropland and grassland land-use intensities (Siebert et al., 2019). Similar experimental work for forests would be a valuable contribution to understanding the vulnerabilities of various soil microbial communities to future climate change. Our study offers the preliminary interpretation that already-degraded soils of croplands will be more vulnerable to climate change than more natural soils (de Vries et al., 2012), representing a major factor that should be explicitly accounted for in future management plans (Griffiths & Philippot, 2013). This suggestion of land cover affecting the vulnerability of microbial communities to other global change drivers is also interesting in light of recent work on the co-occurrence and interaction of multiple biodiversity change drivers, which showed there is spatial overlap, or co-occurrence, between drivers of biodiversity change such as pesticide use, land conversion and climate change (Bowler et al., 2020; Rillig et al., 2019). Therefore, any plan that hopes to manage the consequences of one threat in a given area (e.g., agricultural land use) must also consider the other factors threatening that same area (e.g., climate change). Though Bowler et al. (2020) found that central Europe was not as threatened by climate change as boreal or tundra biomes, temperate European forests were highly affected by a suite of agriculture-related drivers of biodiversity change. Our findings and interpretation also supplement previous studies showing that the response of microbial communities to climate change stressors, such as warming or drought, depends on the established community structure and abiotic conditions (de Vries & Shade, 2013; Griffiths & Philippot, 2013; Tardy et al., 2014). Due to the strong connection between soil carbon cycling and soil microbial properties, we must also consider the effects these climate-driven changes in the microbial community may have on soil carbon dynamics (Cagnarini et al., 2019).

Discovering how a variety of environmental and geographical factors influence soil microbial communities and functioning is an important step to understanding, and thus being able to model, the carbon cycle (Cagnarini et al., 2019; Crowther et al., 2019; Schmidt et al., 2011; Wieder et al., 2013). The present study contributes novel insights into how land cover interacts with these factors and allows us to identify places at higher risk of degradation and climate-change impacts. This identification of higher-risk places can be used to advise management and restoration plans and policy to protect sensitive areas (Guerra et al., 2021). Overall, they indicate that intensive land use alters microbial community functioning related to the carbon cycle, causing the loss of soil carbon and increasing vulnerability to climate change.

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AUTHOR CONTRIBUTIONS

A.O. and A.J. coordinated the LUCAS Soil survey and associated measurements. L.C.S. and A.Lo. performed lab measurements. F.Be. performed the fatty acid measurements. A.H.-B. and G.P. provided the iSBio data for map verification. L.C.S., C.A.G. and N.E. designed the study. L.C.S. and C.A.G. performed the analysis. L.C.S., C.A.G., N.E., S.C. and G.P. interpreted the results with input from all authors. C.A.G. supervised the work. L.C.S. drafted the manuscript with significant feedback and comments from all authors.

DATA AVAILABILITY STATEMENT

The data from this study are available from DRYAD at https://doi.org/10.5061/dryad.g4f4qrfqn. The code can be found at https://codeberg.org/shosh_riv/MicrobialProperties_SOC_paper. Maps are available on the European Soil Data Centre (ESDAC — Panagos et al., 2012) web platform (http://esdac.jrc.ec.europa.eu/).

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BIOSKETCH

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the Supporting Information section.

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