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ORIGINAL RESEARCH ARTICLE

Legacy of land-cover changes on soil microbiology in Burgundy vineyards (Pernand-Vergelesses, France)

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ABSTRACT

Present-day soil physicochemical characteristics, land use/land cover (LULC), and field management practices are commonly recognised as the main drivers shaping archaeal/bacterial and fungal communities in vineyard soils. Few studies have investigated the legacy of past land uses on soil microbial biodiversity, yet anthropogenic disturbances have already been proven to affect soil characteristics over decades. In this study, we explore the possibility of long-lasting impacts of forest-to-vineyard conversion on present-day soil archaeal/bacterial and fungal communities after 15 years of vine cultivation.

The selected study area is in a Burgundian vineyard (Pernand-Vergelesses, Burgundy, France), where it was possible to reconstruct the history of land cover and land use for the past 40 years. Soil samples were collected from five zones managed under similar pedo-climatic conditions but with different land-use histories (a 70-year-old vineyard, a 15-year-old vineyard converted from pine forest, a 15-year-old vineyard converted from mixed forest, a pine forest and a mixed forest). For each zone, basic physicochemical parameters (organic carbon, total nitrogen, copper, C:N ratio, and soil texture) were measured, and DNA was extracted to characterise the microbial biomass, and also the richness and taxonomic composition of archaeal/bacterial and fungal communities (16S and 18S).

Our results show that changes in LULC lead to differential responses in soil microbial biomass, and in archaeal/bacterial and fungal richness and taxonomic composition. After 15 years of cultivation, the present-day microbial biomass and indigenous archaeal/bacterial communities of recent vineyard soils are shown to be partly inherited from past LULC, but no evidence was found of long-term impacts of past land use on fungal communities. Past land-use history should therefore be added to the well-established set of environmental drivers, providing valuable information to explain the spatial variability of soil microbiology, observed at intra-plot, plot, and landscape scales. Integrating the history of changes in LULC is therefore recommended to evaluate and adopt the best strategies to develop sustainable management practices.

KEYWORDS: vineyard soil, land-use history, legacy, bacterial community, fungal community, edaphic properties

INTRODUCTION

Through their vast abundance and diversity, soil microorganisms help to maintain soil fertility and protect soils from physical degradation by increasing soil stability and favouring soil aggregation (Maron *et al.*, 2018). Soil microorganisms play major roles in ecosystem functioning and are strongly involved in major global biogeochemical cycles of soil nutrients such as carbon, nitrogen, and phosphorus (Schimel and Schaeffer, 2012). Soil microbiota have also demonstrated their usefulness, both in the degradation and removal of organic pollutants (Maron *et al.*, 2011), and by their contribution to plant growth (van der Heijden *et al.*, 2008; Mendes *et al.*, 2013). In vineyard contexts, soil microbiology is considered as a factor potentially contributing to wine quality by influencing its organoleptic properties (Bokulich *et al.*, 2014; Zarraonaindia *et al.*, 2015; Liang *et al.*, 2019), although the role of soil microorganisms in terroir expression has not yet been fully explored. Inventorying and analysing the microbial component of soils is crucial, in order to better understand soil functioning, and to adapt management practices for long-term sustainable viticulture (Lemanceau *et al.*, 2015; Burns *et al.*, 2016; Liang et *al.*, 2019; Karimi *et al.*, 2020).

Over the past few decades, with the development of environmental genomics and bioinformatics, substantial research has been conducted at various spatial scales and in various contexts to evaluate the complex linkages between the key factors shaping the diversity and abundance of microbial communities. At a large spatial scale, increasing evidence of differences in microbial vineyard communities from geographically distant vineyard regions supports the idea of a microbial terroir (Bokulich *et al.*, 2014; Coller *et al.*, 2019; Liu *et al.*, 2019). At landscape or plot scale, soil physicochemical characteristics, such as soil organic carbon, pH, and C:N ratio (Zarraonaindia *et al.*, 2015; Zehetner *et al.*, 2015; Terrat *et al.*, 2017; Liang *et al.*, 2019), land use/land cover (LULC), and field management practices (Zehetner *et al.*, 2015; Burns *et al.*, 2016; Coller *et al.*, 2019; Karimi *et al.*, 2020; Di Giacinto *et al.*, 2020) are identified as the most important drivers of microbial biodiversity. In many cases, it has been demonstrated that soil preparation before vineyard plantation and other viticultural practices have altered soil microbial quality in terms of abundance, diversity, and activity (Costantini *et al.*, 2018; Dequiedt *et al.*, 2011; Karimi *et al.*, 2020).

Overall, most of these studies have examined the impact of vineyard soil management practices over short timescales, whereas the long-term effects of past LULC have seldom been explored, although anthropogenic disturbances have been proven to affect soil properties over decades. One such study showed that centennial landscape structure and past management practices continue to affect present-day soil erosion patterns in vineyards (Chevigny *et al.*, 2014). Long-term effects on various soil chemical properties of post-agricultural forests and grasslands have been reported to persist for centuries after agricultural activity had ceased (Koerner *et al.*, 1999; Dupouey *et al.*, 2002). Yet very few studies have investigated the legacy of past management practices and past land use on soil microbial biodiversity (Turley *et al.*, 2020). Some studies have explored the long-lasting effects of past agricultural activities on forest recovery after agricultural abandonment (Stockmann *et al.*, 2015; Katulanda *et al.*, 2018). Conversely, little has been done to assess the effects of the conversion from a natural to a managed agricultural ecosystem on soil microbial diversity (Lynn *et al.*, 2017). One question that arises in vineyard contexts is whether present-day soil biodiversity may also be inherited from LULC changes. Herein, we intend to assess this hypothesis by focusing on two key questions:

- Is there any evidence of a persistent effect of past forest land use on present-day microbiology in Burgundian vineyard soils?

- Is soil microbiota more related to edaphic properties than to past and/or present-day land use?

One of the main challenges is to disentangle the individual contributions of each of the driving environmental factors (soil, climate, topography, and parent material) and field management practices. Our study presents an original approach to overcome these difficulties by tracking biodiversity indicators at a local spatial scale in Burgundian vineyard and forest soils with different land-cover histories, thus eliminating major differences in other factors (parent material, topography, climate, and vine cultivar). Such an opportunity is rare in historical Burgundy, where vines have often been cultivated for centuries in most vineyard plots. The study area, composed of five zones with different LULC characteristics, will be presented first, followed by a description of the methods used. The results from the analysis of physicochemical parameters, microbial biomass, and the diversity and taxonomic composition of indigenous archaeal/bacterial and fungal communities will then be described and discussed in relation to the LULC history of each of the five zones.

MATERIALS AND METHODS

1. Study area

Fieldwork was carried out on the slopes of the Pernand-Vergelesses vineyards (Côte de Beaune, Burgundy, France; Figure 1A). The vineyard plots (47.06°N, 4.84°E), which cover the entire slope, are bordered upslope by a limestone plateau covered by mixed forest (Figure 1B,C). The climate is temperate, warm and dry in summer, rainy in fall and winter. The mean annual precipitation ranges from 700 to 900 mm/yr.

The hillslope morphology is slightly convex, with a slope ranging from 16° to 18°. The silty–clayey soils, developed on Jurassic marls, are classified as Calcaric Cambisol (IUSS Working Group WRB, 2015). Soil samples were collected from two adjacent vineyard plots (*En Caradeux* and *Creux de la Nêt*) on these slopes,

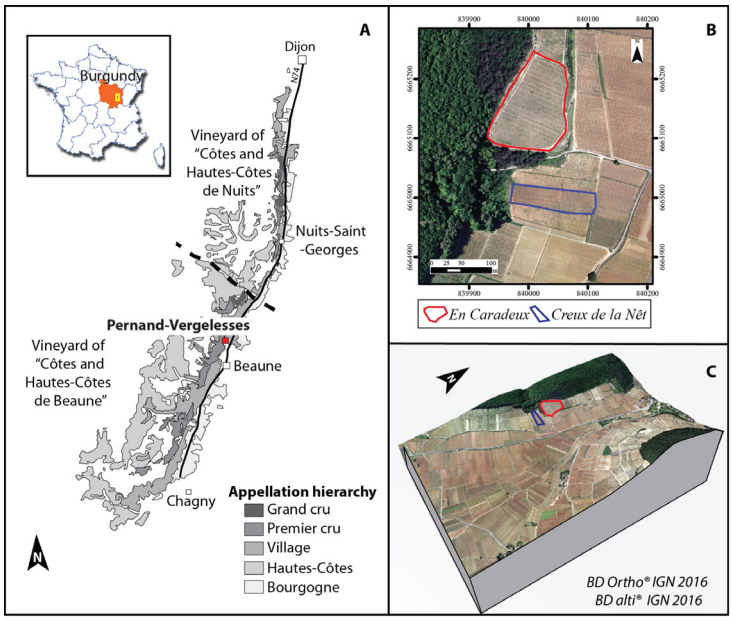


FIGURE 1. Location of the study area, showing the two vineyard plots: *En Caradeux* in red and *Creux de la Nêt* in blue.

separated by a pine forest (Figure 1B,C). The vines of the *Creux de la Nêt* plot were planted in 1940 (or even earlier), whereas the vines of the *En Caradeux* plot were planted in 2002, after tree removal and deep soil ripping. In both plots, the rows are oriented in the direction of the steepest slope, with a 1 m (row) × 1 m (vine) spacing for vine stocks. According to the owners, the soil is not tilled. The *En Caradeux* plot was entirely grass-covered between 2006 and 2015 to protect the soil from erosion processes (Figure 1B). Since 2015, the inter-rows have been continuously under grass cover, but the rows are weeded. In the *Creux de la Nêt* plot, the rows are chemically weeded.

2. Land-use/Land-cover history reconstruction

LULC maps of the study area were reconstructed to assess any significant changes over the past 40 years, from 1977 to 2016 (Figure 2). To this end, we downloaded a series of aerial photographs (1940, 1977, 1985, 2000, 2002, 2006, 2010, 2016) from the open-source data website of the French Institut Géographique National (IGN; https://remonterletemps.ign.fr) covering the study period, with a baseline in 1940. These historical photographs were georeferenced using anchor points recognisable in the ortho-photograph also available from the IGN website (https://geoservices.ign.fr/). Land-cover maps were then completed by interpreting aerial images and identifying various types of soil cover (e.g., vineyard, forest, shrub, and bare soil).

Among the historical images, the infrared colour photograph acquired in 2000 was particularly useful to distinguish between pine and mixed forests. Aerial image analysis revealed that:

- The plateau is covered today by a mixed forest. The 19th-century French military map and the 18th-century Cassini map (https://geoportail.gouv.fr) together indicate continuous forest cover in this part of the study area for at least the past 250 years. Parts of the plateau were occasionally used for ovine pasture in medieval and modern times.

- The *Creux de la Nêt* plot has been continuously planted with vines from at least 1940 onwards and was also indicated as a vineyard plot on the two ancient maps.

- From 1977 to 2002, the southern part of the *En Caradeux* plot was covered by a pine forest planted after World War II, while the northern part was covered by a spontaneous mixed forest. In 2002, the plot was cleared of trees before vines were planted, as shown in the aerial photograph. No further modifications in land use have been identified after 2002.

3. Soil sampling and data analysis

3.1. Soil sampling strategy

Soil samples were collected with an auger, from five adjacent zones, in May 2017 (Figure 3). The first reference zone (Old Vineyard or OV) was in *Le Creux de la Nêt*, a plot cultivated as a vineyard for more than 70 years (Figure 3 A,B). The land cover of the next two zones, both in the *En Caradeux* plot, more recently converted to vineyard use, was previously a pine forest (VPF) in the south of the plot, and a mixed forest (VMF) in the north of the plot (Figure 3B). The last two zones, used for reference soil values (together with the OV zone), were both under forest cover (Figure 3A,B): one is mixed forest (MF), and the other is pine forest (PF). For each of the five zones sampled in the study, the soils developed on the same parent material and under similar climate conditions. Six soil samples were collected in the 0–20 cm topsoil layer and were bulked to obtain a composite sample for each zone. For each of the two forest zones, samples were randomly collected over an area of about 20 m^2. For each of the three vineyard zones,

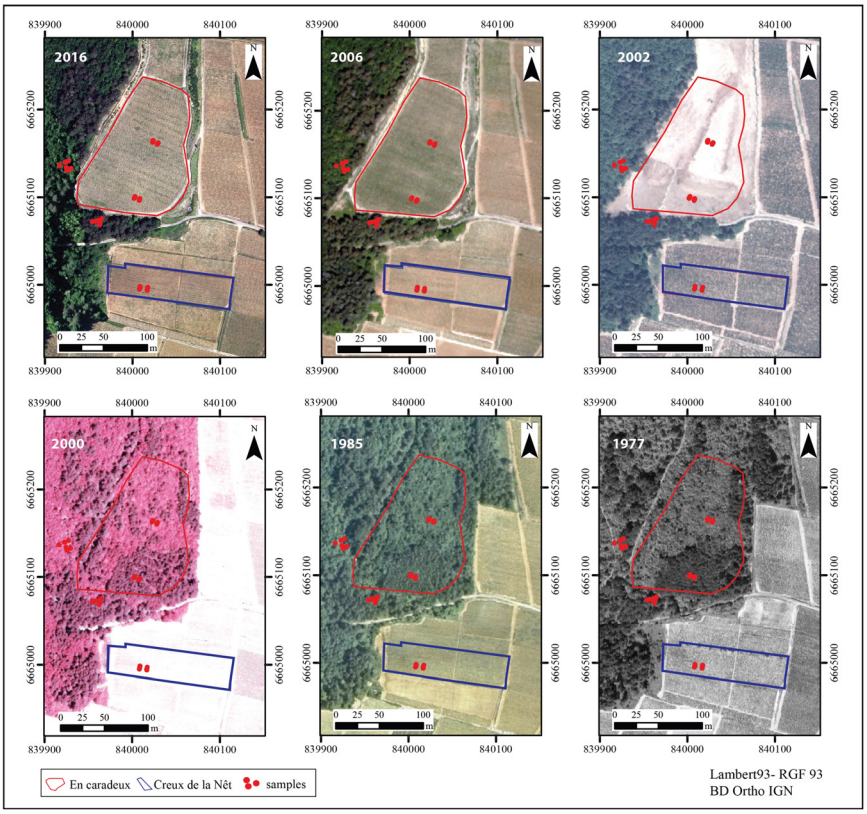


FIGURE 2. Land use/land cover (LULC) history of the study area from 1977 to 2016 (top left is most recent, bottom right is most ancient).

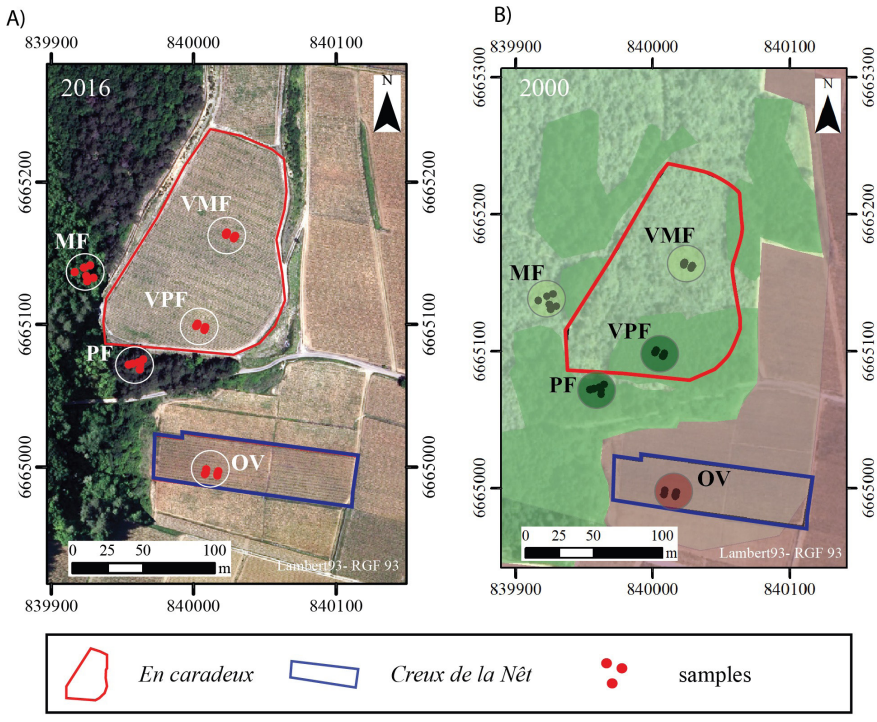


FIGURE 3. A) Location of the samples collected, showing present-day land use/land cover (LULC); B) Location of samples collected, showing historical LULC.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

two samples were collected from the same row, 6 m apart, for 3 contiguous rows. All 30 soil samples were gently air-dried, then sieved to 2 mm. Part of each sample was lyophilised and stored at –40 °C, before microbiological analysis.

3.2. Soil physicochemical analyses

Basic soil physicochemical properties were measured for each composite soil: particle-size distribution, pH, organic carbon (Corg), total nitrogen (Ntot), $CaCO_3$, C:N ratio, and Cu (mg/kg). Physical and chemical analyses were performed by the Soil Analysis Laboratory of INRA (ARRAS, France, LAS) using standardised methods (https://www6.hautsdefrance.inrae.fr/las/Methodes-d-analyse). Grain-size distribution was obtained using sieve size for sand fraction (63 µm–2 mm), silt fraction (2 µm–63 µm), and clay fraction (< 2 µm), following the French norm NF P18-560. Total carbonate content was estimated after decomposition of the $CaCO_3$ contained in the soil by hydrochloric acid. Carbon and total nitrogen contents were determined by dry combustion under oxidising conditions and dosage of CO_2 and N_2 released by gas chromatography, following the standard norm NF ISO 10694. The organic carbon content was obtained by correcting from the total carbonate content (NF ISO 10963).

3.3. Microbial community characterisation

3.3.1. Soil DNA extraction and molecular microbial biomass quantification

Soil DNA was extracted and purified using the procedure described by Plassart *et al.* (2012). This protocol is based on three specific steps: (i) microbial cell lysis by chemical and physical action; (ii) deproteination; (iii) alcohol precipitation and washing of extracted nucleic acids. Each soil sample was placed in a 15 ml Falcon tube and mixed with 4 ml of a solution containing 100 mM Tris (pH 8.0), 100 mM EDTA (pH 8.0), 100 mM NaCl, and 2 % (wt/vol) sodium dodecyl sulphate. Then 2 g of 100 µm silica beads, 2.5 g of 1.4 mm ceramic beads, and 4 glass beads (4 mm in diameter) were added to the mixture. Samples were homogenised for 3 × 30 s at 4 m.sec-1 in a FastPrep H-24 (MP-Biomedicals, NY, USA). The homogenised samples were incubated for 30 min at 70 °C, then centrifuged at 7,000× *g* for 5 min at 20 °C. To remove proteins from the extracts, 1 ml of supernatant was incubated for 10 min on ice with 1/10 volume of 3 M potassium acetate (pH 5.5), then centrifuged at 14,000× *g* for 5 min. Finally, after precipitation with one volume of ice-cold isopropanol, nucleic acids were washed with 70 % ethanol. Crude DNA extracts were quantified by gel agarose electrophoresis, stained with ethidium bromide, using calf thymus DNA to produce a standard curve, to provide a reliable estimate of soil microbial molecular biomass (Dequiedt *et al.*, 2011). After quantification, crude DNA samples were purified on PVPP (polyvinylpolypyrrolidone) Microbiospin minicolumns (Bio-Rad). The eluates were then collected and purified to remove residual impurities using the Geneclean Turbo kit (MP-Biomedicals, NY, USA). The purified DNA extracts were quantified using the Quantifluor staining kit (Promega, Lyon, France).

3.3.2. High-throughput sequencing of 16S and 18S rRNA gene sequences

For archaeal/bacterial diversity, a 16S rRNA gene fragment of about 430 bases was amplified from each DNA sample with the following primers: F479 (5'-CAG CMG CYG CNG TAA NAC-3') and R888 (5'-CCG YCA ATT CMT TTR AGT-3'), with PCR conditions as previously described (Terrat *et al.*, 2015).

For fungal diversity, a 350-base 18S rRNA gene fragment was amplified from each DNA sample with the following primers: FF390 (5'-CGA TAA CGA ACG AGA CCT-3') and FR1 (5'-ANC CAT TCA ATC GGT ANT-3') (Chemidlin Prévost-Bouré *et al.*, 2011), with PCR conditions as previously described (Terrat *et al.*, 2015). All PCR products were purified using the Agencourt® AMPure® XP kit (Beckman Coulter, Milano, Italy) and quantified with the Quantifluor (Promega, Lyon, France) staining kit, according to the manufacturer's instructions.

A second PCR was performed with the purified PCR products as matrix (7.5 ng of DNA for bacteria and archaea, and 5 ng of DNA for fungi), to add 10 bp multiplex identifiers to the 5′ and 3′ ends of the primers, for the specific identification of each sample. For bacteria and archaea, the second PCR conditions were the same as previously described, but with only seven cycles. For fungi, the second PCR conditions were optimised with seven cycles, with denaturation at 94 °C for 1 min. The PCR products were purified with the MinElute PCR purification kit (Qiagen NV) and quantified with the Quantifluor (Promega, Lyon, France) staining kit, according to the manufacturer's instructions. Equal amounts of each sample were pooled and then cleaned with the SPRI (Solid Phase Reversible Immobilization) method using the Agencourt® AMPure® XP kit (Beckman Coulter, Milano, Italy). Finally, the pooled samples were sequenced with an Illumina MiSeq instrument (Illumina Inc, San Diego, CA, USA) operating with V3 chemistry and producing 300 bp paired-end reads.

3.3.3. Bioinformatic analyses

Bioinformatic analyses were performed using the BIOCOM-PIPE, which was initially developed by the GenoSol platform (INRAE, Dijon, France) (Terrat *et al.*, 2012) and recently published (Djemiel *et al.*, 2020). First, 16S and 18S rRNA raw reads (respectively 995,700 and 948,211) were sorted according to each sample using multiplex identifiers. Low-quality reads were then deleted based on their length (less than 350 bp for 16S reads, and less than 300 bp for 18S reads), their number of ambiguities and their primer(s) sequence(s). Then, after rigorous dereplication (i.e., clustering of strictly identical sequences), the dereplicated reads were aligned using Infernal alignment (Nawrocki and Eddy, 2013) and clustered into operational taxonomic units (OTU). This clustering is based on a greedy strategy in which OTUs are constructed incrementally by comparing an abundance-ordered list of input reads against a representative set of already chosen sequences, with a defined sequence similarity threshold of 95 %. A filtering step based

on the "hunting-recovering" concept (Djemiel *et al.*, 2020) was then carried out to check all singletons (reads detected only once and not clustered, which might be artefacts, such as PCR chimeras) based on the quality of their taxonomic assignments.

Finally, to compare the datasets efficiently and avoid biased community comparisons, the reads retained were homogenised by random selection of 10,000 reads for 16S and 18S rRNA gene sequences. The high-quality reads retained were used for: (i) taxonomy-independent analyses, determining richness index using the OTU dataset; (ii) defining global OTU matrices using ReClustOR (Terrat *et al.*, 2020), a post-clustering tool that improves the reliability of OTU-based results and analyses based on French RMQS biological reference databases of archaeal/bacterial communities (Terrat *et al.*, 2017) and fungal communities (data not shown); (iii) taxonomy-based analysis using similarity approaches against curated reference databases from SILVA r132 (Quast *et al.*, 2013). The raw datasets are available on the EBI database system under project accession number PRJEB50819.

3.4 Statistical analyses

The effect of management practices and LULC history on soil chemical and molecular microbial biomass was evaluated by the non-parametric Kruskal–Wallis test. Differences between means were tested by paired multiple comparisons with Bonferroni correction. Microbial diversity indices were compared by analysis of variance (ANOVA, multiple pairwise comparisons), and significant differences between the five zones were identified by Tukey's HSD ($P < 0.05$).

All the statistical analyses were performed in the R environment (v4.0.1) (R Core Team, 2021).

Taxa (phyla) data were organised in a data matrix, which was subjected to principal component analysis (PCA) on a correlation matrix using the ADE-4 software (Thioulouse *et al.*, 1997). This method ordinated the samples on a factorial map based on the scores of the first two principal components. The corresponding arrows for bacterial and fungal phyla were drawn to highlight phyla with contributions above average.

We used linear discriminant analysis effect size (LEfSe) to detect taxa (whatever the taxonomic level) with significant differential abundance, for each soil with a different LULC history (Segata *et al.*, 2011). The LEfSe method is based on (i) a non-parametric Kruskal–Wallis rank sum test to search for statistically different microbial groups (both 16S and 18S), followed by (ii) linear discriminant analysis (LDA scores > 3.9 for 16S and > 4 for 18S) to assess the effect size of each differentially abundant group.

Both the importance of the environmental variables and the variance explained by the models were estimated using the rda and ordistep functions in the R package vegan (Oksanen *et al.*, 2013).

RESULTS AND DISCUSSION

1. Soil physicochemical characteristics

Figure 4 presents the soil physicochemical characteristics for the five zones studied. The soils appear quite similar, with high $CaCO_3$ values, a clayey-sandy silty texture, and alkaline pH values, confirming that they developed on the same marl/limestone geological substrate. These values are very similar to physicochemical analyses performed on soils collected in the upper hillslopes of the Burgundian vineyards (Brillante *et al.*, 2014; Chevigny, 2014).

Interestingly, some finer chemical differences can be highlighted between the five soils. Specifically, we observed similar trends in the values for organic carbon and nitrogen content, which are among the most important physicochemical parameters describing the quality and functioning of the soil, and shaping the diversity and abundance of microbial communities (Stockmann *et al.*, 2015; Zehetner *et al.*, 2015; Terrat *et al.*, 2017; Liang *et al.*, 2019). The forest soils (PF and MF) have the highest Corg content (and Ntot), with heterogeneous but comparable values ranging from 4.9 to 6.7 %. In contrast, the OV soils have the lowest and most homogeneous Corg content (and Ntot), with values more than six times smaller (around 0.96 %). The average levels in the VPF and VMF soils (respectively around 2.05 and 2.44 %) after 15 years of vine growth are in an intermediate position between those measured in the OV soils and those from the PF and MF soils. The distributions of median values of Corg for these sites are statistically different, except between MF and PF. The Kruskal–Wallis test appeared to be significant ($\alpha < 2.1\ 10^{-15}$), meaning that at least one site is different from the others. Pairwise comparisons between groups indicate significant differences for all pairs, except between PF and MF ($p = 0.06$). When sites are ranked from the highest to the lowest values, the following order is obtained: MF~PF, VMF > VPF > OV. This result indicates that VPF and VMF values belong to a linear trend between OV and the two forest soils, MF and PF. Reductions in Corg and Ntot pools between forested and old vineyard soils can be attributable to the conversion to cropland; they probably result from a progressive depletion over time in the stock of organic matter due to continuous cultivation, soil tillage, and limited grass cover. This hypothesis is consistent with the results of studies reported by Guo and Gifford (2002), Guigue *et al.* (2015) and Karimi *et al.* (2020). The intermediate levels observed in the VMF and VPF soils could reflect a legacy of the organic stock from the former forest. This result might also be partly explained by the present-day grass cover in the VMF and VPF inter-rows, which could increase the Corg content compared to the OV inter-row, which is chemically weeded (Abad et *al.,* 2021).

The values of the C:N ratio follow the same overall increasing trend as that observed for Corg and Ntot contents. The lower values (ranging from to 10 to 12.5) are associated with the vineyard soil from the OV zone, matching those expected for arable soils in the Burgundy region (Guigue *et al.*, 2015). Conversely, forest soils exhibit higher values, which are

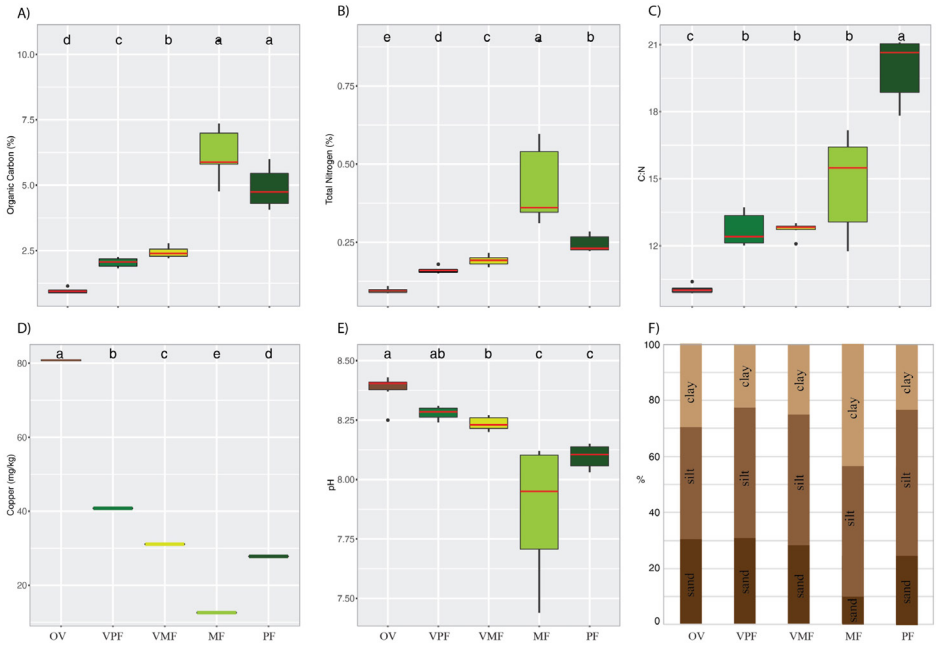


FIGURE 4. Soil physicochemical characteristics for the five zones.

Organic Carbon; B) Total Nitrogen; C) C:N ratio; D) Copper; E) pH; F) Soil texture.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

Error bars represent the standard deviation of six independent replicates. At the top of the figure, different lowercase letters indicate significant differences across zones, based on the Kruskal–Wallis test with Bonferroni correction.

consistent with regional measurements from Burgundian forests, compiled in the French Soil Quality Monitoring Network (RMQS) (Dequiedt *et al.*, 2011). Higher values in PF compared to MF soils have also been observed at the European spatial scale (Cools *et al.*, 2014). As already shown for the organic carbon content, C:N ratios display intermediate values for VMF and VPF, falling between those measured in OV and those measured in PF and MF. This could also indicate that the Corg value is the legacy of the initial organic stock of the former forest, which has not yet decomposed.

We found the opposite trend for pH values between OV and PF. The soil pH values, ranging from 7.7 to 8.5, are significantly higher in the cultivated vineyard soils than in the forest soils (MF and PF).

The levels of Cu were very low in the forest soils (< 30 mg/ kg) and are, in general, higher in the vineyard soils (30 mg/kg to 80 mg/kg), due to yearly repeated applications of Cu-based fungicides. Our results also indicate that the levels of Cu are significantly higher in the old vineyard, OV, compared to the recent vineyard zones, VPF and VMF, which could indicate a legacy effect from times when higher recurrent application rates of Cu fungicides were common. Nevertheless, all Cu levels remain relatively low, probably too low to significantly alter soil microorganisms in terms of biomass and diversity (Karimi *et al.*, 2021). Altogether, our results reveal significant differences in soil properties that can be explained as a function of the zone's LULC history, confirming that Corg, Ntot, pH, Cu, and C:N ratio are the prevailing factors in soils that explain the differences observed between the five zones studied. Forest soils exhibit the highest organic carbon and nitrogen levels, the highest C:N ratio, and the lowest pH values. In OV soils, Corg and Ntot pools and C:N ratios are all significantly lower than in other soils. All the chemical properties of the recent vineyard soils (VMF and VPF) present intermediate values that fall between those of OV and those of forest soils. The accumulation of Cu confirms that the two vineyard plots reflect distinct histories of viticultural practices. Finally, our analyses suggest that there is probably a legacy effect of the former forest on the soil resource availability of the recent vineyard soils, which can impact the habitat quality of microorganisms and the trophic resources.

2. Microbial biomass and diversity in soils associated with different LULC histories

An overview of soil molecular microbial biomass, and bacterial and fungal taxonomic richness is reported in Figure 5. The results of the variation partitioning analysis are reported in Table 1.

The old vineyard plot (OV) cultivated since at least 1940 (*Le Creux de la Nêt*) displays the lowest microbial biomass (around 35 mgDNA.g^{-1}soil), whereas samples collected from pine and mixed forest soils (PF and MF) are 5 to 6 times higher (respectively around 140 mgDNA.g^{-1}soil and 220 mgDNA.g^{-1}soil) (Figure 5A). More precisely, the highest C:N ratio observed in the pine forest might partly explain the lower molecular microbial biomass observed for this zone compared to the mixed forest. The microbial biomass

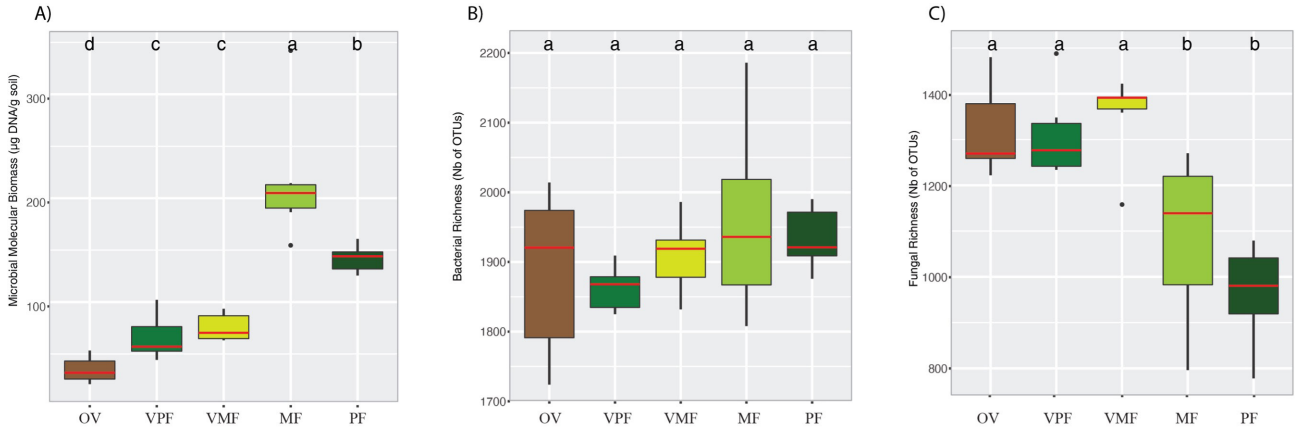


FIGURE 5. Microbiological characteristics for the five zones.

A) Microbial biomass; B) 16S richness; C) 18S richness

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

Error bars represent the standard deviation of six independent replicates. At the top of the figure, different lowercase letters indicate significant differences across zones, based on Tukey's HSD test ($P < 0.05$).

of *En caradeux* plot (VPF and VPF) exhibits intermediate values (60 to 80 mgDNA.g^{-1}soil), which are higher than those usually measured in the French national vineyards referenced in the RMQS soil survey (around 25 mgDNA.g^{-1}soil, Horrigue *et al.*, 2016). The roles of environmental filtering on soil microbial molecular biomass and microbial diversity (richness) were evaluated. The results of the variation partitioning analysis show that organic carbon and copper were the most important drivers of soil microbial molecular biomass (OC with a positive effect and Cu with a negative effect), explaining more than 89 % of the total variation (Table 1). Overall, our analyses show that the values of microbial molecular biomass follow the same overarching trend described for the soil organic carbon content. We suggest that the recent forest history of the vineyard plot *En Caradeux* provides a favourable habitat for microorganisms because of its more stable and abundant organic matter, a relict of the previous forest cover. Organic matter content provides nutritive resources for microorganisms and is one of the main drivers of soil microbial biomass (Dequiedt *et al.*, 2011).

Measurements of bacterial taxonomic diversity do not highlight significant differences between the soils of the five zones (Figure 5B). This suggests that bacterial taxonomic diversity was not different between forest and vineyard soils and is not as clearly related to LULC history as the soil microbial biomass, even if a positive trend can be observed. This result is not surprising since bacterial taxonomic richness is generally found to be at equivalent levels or higher in cropland soils compared to grassland or

TABLE 1. Variation partitioning analysis.

	Environmental variables	% Variable explained	Effect
Microbial molecular biomass	Organic carbon	13.3	Positive
	Copper	7.8	Negative
	Interaction	67.9	
	Total	89	
	Residuals	11	
16S Richness	Organic carbon	38.6	Positive
	Sand	12.3	Positive
	Interaction	0	
	Total	50.9	
	Residuals	49.1	
18S Richness	C:N	41.5	Negative
	Total	41.5	
	Residuals	58.5	

forest soils (Terrat *et al.*, 2017). After variation partitioning analysis, organic carbon and sand were the most important drivers of archaeal/bacterial richness, with a positive effect for both, explaining 50.9 % of the total variation with interactions (Table 1). These results are consistent with other studies demonstrating that bacterial taxonomic richness is mostly influenced by soil texture and pH (Rousk et *al.*, 2010; Terrat *et al.*, 2017), whereas the LULC legacy in our study is mainly correlated with soil organic carbon content and C:N ratio between zones.

Conversely, we observed a lower fungal taxonomic richness in forest soils when compared to vineyard soils (Figure 5C). This observation has been previously demonstrated between croplands and forests (George *et al.*, 2019) and could be induced by differences in the degree of environmental disturbance between vineyard and forest contexts. In forests, the low-disturbance environment could cause competitive exclusions and induce the dominance of opportunistic organisms. In vineyard contexts, higher soil disturbance could limit the process of competitive exclusion and thus favour higher diversity, as predicted by the "humped-back" model describing the response of the diversity of a community to environmental stress (Giller *et al.*, 1998), and also observed with regard to bacterial richness in agricultural soils (Terrat *et al.*, 2017). This greater fungal alpha-diversity in croplands than in forests could be attributed to greater nutrient availability from fertiliser inputs in arable soils, promoting some microbial species (Szoboszlay *et al.*, 2017). As with molecular microbial biomass, the C:N ratio appears to be the most important environmental variable, explaining 41.5 % of fungal diversity (Table 1). Our results are in accordance with previous studies indicating the same trend (George *et al.*, 2019). Fungal diversity does not appear to be significantly influenced by LULC history but rather by present-day edaphic properties.

3. Archaeal/bacterial and fungal taxonomic composition of soils associated with different LULC histories

Overall, *Proteobacteria*, *Actinobacteria*, *Acidobacteria*, *Planctomycetes*, and *Chloroflexi* were the most abundant bacterial phyla identified in our dataset, with *Thaumarchaeota* the most abundant of the Archaea (Figure 6A).

In all soils, *Proteobacteria* dominated, followed by *Bacteroidetes*, and together they accounted for more than 50 % of the total abundance. However, the relative abundances of these phyla varied as a function of the LULC history. The relative abundance of *Thaumarchaeota* and *Chloroflexi* followed the opposite trend to that of *Planctomycetes*. The relative abundance of *Thaumarchaeota* and *Chloroflexi* exhibited higher values in OV soils (respectively 24.4 % and 3.7 %) than in MF soils (respectively 1.9 % and 1.2 %) and PF soils (respectively 1.2 % and 1.1 %).

Conversely, the relative abundance of *Planctomycetes* was lower in OV (5.3 %) than in MF (11.1 %) and PF soils (12.3 %). An additional PCA analysis was performed to assess the influence of the land-cover changes on soil archaeal/bacterial communities at the phylum level. The PCA analysis revealed clear significant differences in bacterial taxonomic composition between soil samples from the five zones, forming clusters related to the three LULC histories, i.e. forest, old vineyard, and recent vineyard (Figure 6B).

The bacterial composition of MF and PF forest soils was characterised by a higher relative abundance of *Gamma-Proteobacteria*, *Bacteroidetes*, and *Planctomycetes* than for OV. This microbial community structure is favoured by soils displaying high availability of Corg, a high C:N ratio (e.g., *Alpha* and *Gamma-Proteobacteria)*, low pH (e.g., *Alpha-Proteobacteria* and *Planctomycetes*), and fine soil texture (e.g., *Bacteroidetes*), the edaphic factors described for the forest soils, PF and MF. This observation is also consistent with the microbial community

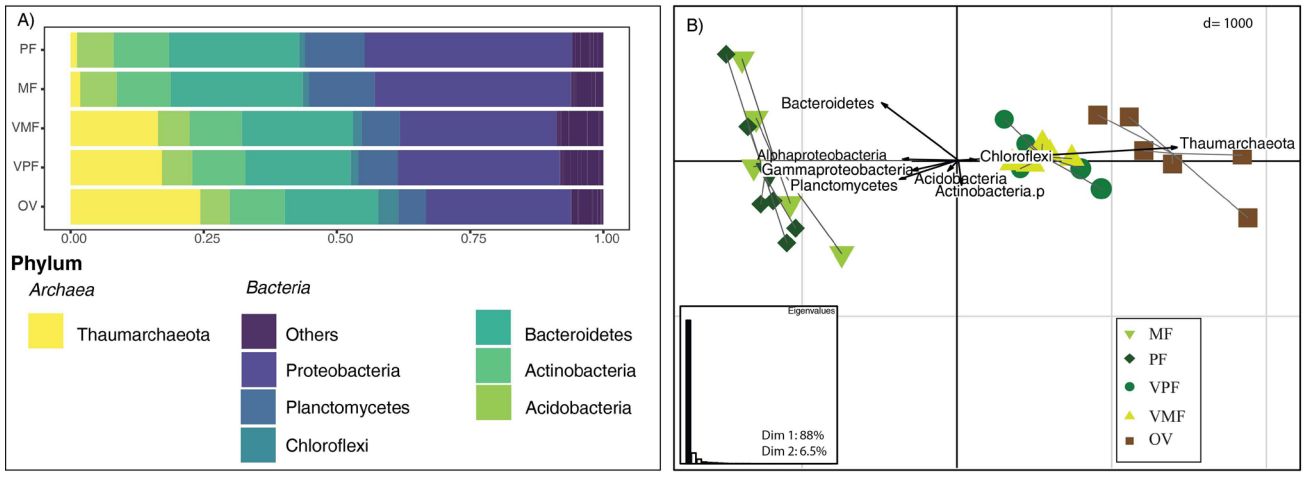


FIGURE 6. A) Relative abundance of major archaeal and bacterial communities; B) Soil archaeal/bacterial taxonomic composition for the five zones.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

structure predicted for forest contexts and referenced in the RMQS national soil survey (Karimi *et al.*, 2018). The archaeal/ bacterial community composition of the old vineyard soil ecosystem exhibited a different signature, dominated by the phylum *Thaumarchaeota*, which has also been identified in cropland soils defined by a positive effect of pH, and negative correlation with organic carbon content (Karimi *et al.*, 2018).

Finally, Figure 6B demonstrates that the microbial communities of the *En Caradeux* plot were compositionally distinct from the two cases described above. Soil samples display an intermediate taxonomic signature based on a combination of the dominant phyla in forest soils and those detected in old vineyard soils. We suppose that this signature was not indicative of any ecological coherence, but might rather indicate transitory processes shifting from a forest to a vineyard microbiome composition.

Regarding fungal phyla, the results of our analyses revealed that the fungal communities are dominated by three phyla (*Ascomycota*, *Basidiomycota*, and *Mucoromycota*), while 17 %, on average, were composed of low-abundance OTUs (Figure 7A). *Basidiomycota* (70 %) are predominant in the MF and PF forest soils, whereas *Ascomycota* and *Mucoromycota* abundances were higher in vineyard soils compared to forest soils. By contrast with results for bacteria, PCA analysis divides fungal communities into only two categories (Figure 7B): all vineyard soil samples (OV, VMF, and VPF) were separated from forest soil samples (MF and PF).

These findings are consistent with previous studies showing that, at the phylum level, *Basidiomycota* are predominant in forest environments, whereas *Ascomycota* and *Mucoromycota* prevail in agricultural lands and grasslands (George *et al.*, 2019; Navarro-Noya *et al.*, 2021). This result is in agreement with previous studies reporting more *Ascomycota* in croplands than in forests, with the opposite trend for *Basidiomycota* (Tedersoo *et al.*, 2014; Liu *et al.*, 2020). These two categories reflect environmental conditions differing in pH values and in their ability to degrade a more or less recalcitrant organic matter (Rousk *et al.*, 2010; Tedersoo *et al.*, 2014). Accordingly, by contrast to what has been observed in bacterial communities, we suppose that the legacies of land-use history fade away rapidly, and that fungal communities are predominantly influenced by present-day soil chemical properties.

DISCUSSION

Ultimately, the purpose of this study was to explore the possible relationships between LULC history and present-day soil biodiversity by examining the long-lasting impacts of a forest-to-vineyard conversion after 15 years of cultivation on soil microbial communities.

In our case study, the results obtained suggest that LULC changes lead to differential responses in soil microbial biomass, and bacterial and fungal richness and taxonomic composition, which was illustrated by LEfSe analysis (Figures 8 and 9).

Figure 8 shows that the Archaea domain discriminated exclusively the old vineyards, in particular the phylum *Thaumarchaeota* and family *Nitrososphaeraceae* with two genera of *Candidatus* (Candidatus_Nitrososphaera and Candidatus_Nitrocosmicus). These bacteria are known to oxidise nitrite to nitrate, suggesting a community more specialised for nitrogen cycling in OV soils (Hang *et al.*, 2021). We also identify the *Chloroflexi* phylum significantly associated with the OV soils, previously pinpointed as an abundant phylum in vineyard soils (Gobbi *et al.*, 2020). There is a greater number of specific

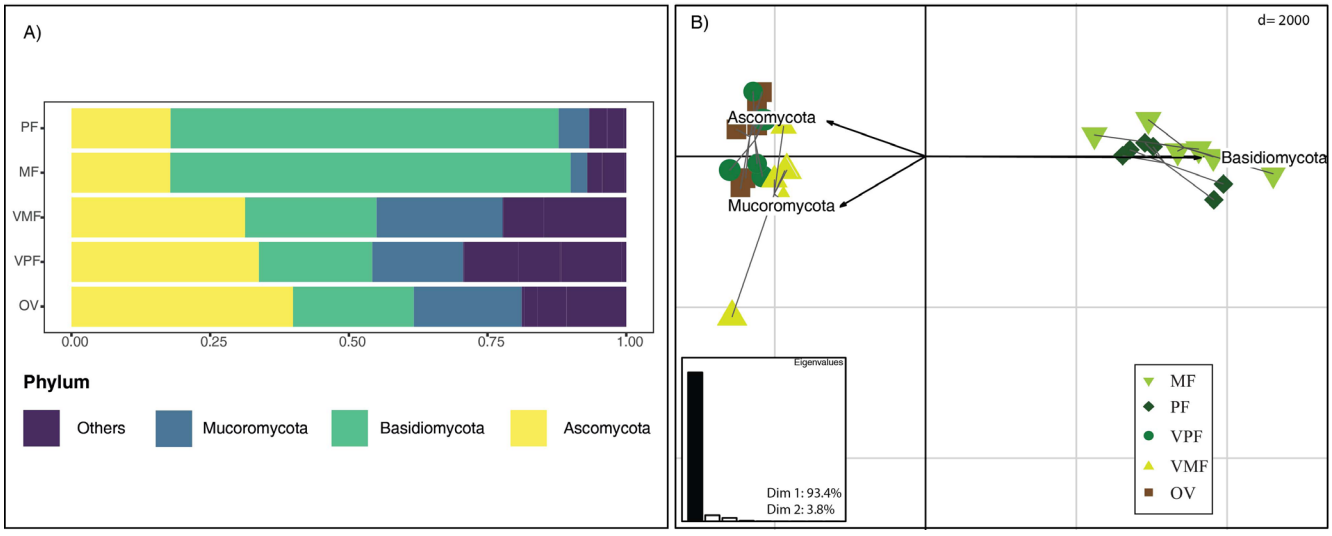


FIGURE 7. A) Relative abundance of major fungal communities; B) Soil fungal taxonomic composition for the five zones.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

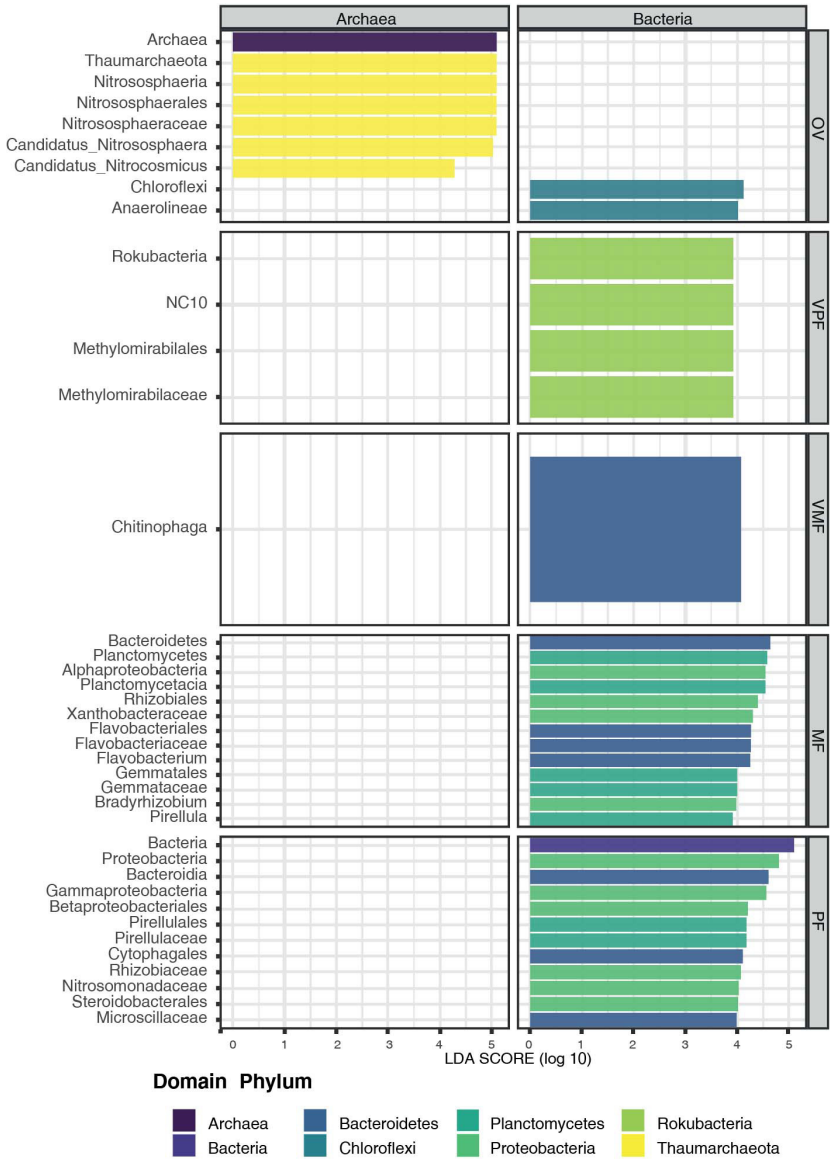


FIGURE 8. Results of LEfSe for archaeal/bacterial taxonomic composition.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

bacterial taxa across various phyla in the forest soils (MF and PF) than in the soils recently converted to vineyard use (VPF and VMF). These results suggest that there is a gradual transition from bacterial to archaeal communities in vineyard soils.

The pattern for fungal diversity is different from that of bacterial diversity; there are more specific taxa for each zone (Figure 9). In forest soils, all significantly abundant taxa belong to the phylum *Basidiomycota* except for one (*Eurotiales* in PF).

Basidiomycota are well known to carry a large range of CAZymes, allowing them to degrade all recalcitrant carbon compounds, particularly in forest soils (Hesse *et al.*, 2015). The *Mucoromycota* members are significantly associated with OV and VMF soils, whereas the phyla *Chytridiomycota* and *Cryptomycota* are discriminant in VPF soils. The *Ascomycota* taxa are more abundant in vineyard soils (whether old or recently converted). This signature with *Ascomycota* as the dominant phylum has previously been observed in vineyard soils (Coller *et al.*, 2019). This result indicates that fungal communities tend to reflect present-day LULC, while bacterial and archaeal communities bear witness to LULC history.

This study shows the persistence of legacy effects on soil microbial biomass and on bacterial taxonomic composition after 15 years of cultivation. By contrast, no evidence of any long-term impact of past land-use changes was detected in fungal communities, for richness and taxonomic composition indicators. These differential responses to LULC history in soil microbiology could be induced by hierarchical effects occurring between present-day and remnant edaphic

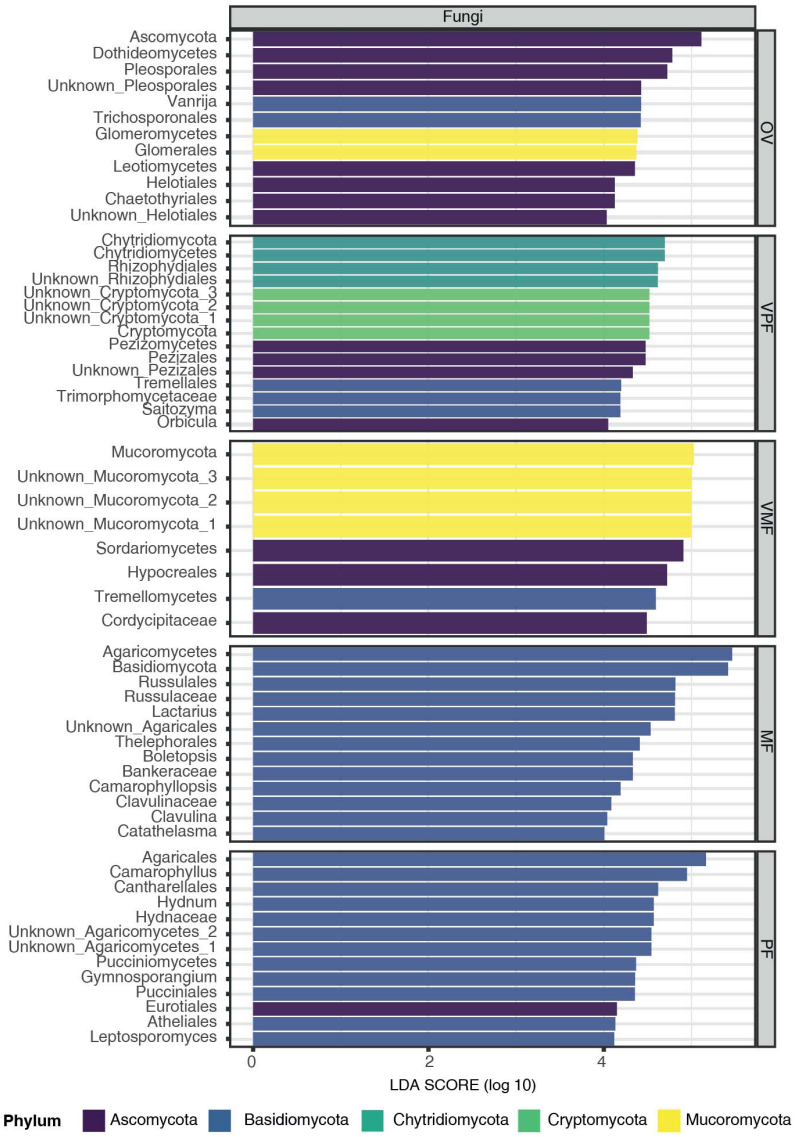


FIGURE 9. Results of LEfSe for fungal taxonomic composition for the five zones.

(OV) *Creux de la Nêt*, an old vineyard plot cultivated continuously for more than 70 years; (VPF) vineyard planted in 2002, previously covered by pine forest; (VMF) vineyard planted in 2002, previously covered by a mixed forest; (MF) mixed forest; (PF) pine forest.

properties, which also differentially shape soil microbial biomass, richness, and taxonomic composition.

The legacy of LULC history on microbial biomass in a recent vineyard plot demonstrated here is not surprising, as it is strongly related to the organic carbon content, a relict of the initial organic stock of the former forest, which has not yet been totally degraded after 15 years.

Unexpectedly, we show that past land use continues to play an important role in shaping present-day archaeal/bacterial taxonomic composition. The resulting taxonomic composition has benefitted from the soil available resources still offered by remnant organic carbon content, but will certainly converge over time towards a vineyard ecosystem.

Our study also suggests that soil fungal richness and taxonomic composition are mainly shaped by present-day land-use and edaphic properties, overwhelming any past land-use effects. This result is in accordance with numerous studies demonstrating that soil pH is a key factor in explaining the fungal community under any environmental conditions.

CONCLUSION

This study presents a novel approach to investigate the long-term effects of LULC history on soil microbiology by comparing zones that were managed under similar pedo-climatic conditions, but with different LULC histories. Soils recently converted into vineyards were compared with a vineyard soil cultivated for more than 70 years, and with two different forest soils. The results from this study show

that, after 15 years of cultivation, the present-day microbial biomass and indigenous bacterial communities of recent vineyard soils were partly inherited from past LULC.

These preliminary results are promising, even if they remain too limited to allow any definitive conclusions to be drawn. This study confirms the relevance of examining the microbial signature of historical soil land use and its possible legacy. These findings indicate that LULC history should be integrated into the well-established set of environmental drivers. It could help to explain the spatial variability of soil microbiology, observed at the intra-plot, plot, and landscape scales. Ignoring the impact of legacy LULC on soil microbiology may hinder our capacity to fully assess how biodiversity responds to changes in land use and management practices, with the resulting consequences for long-term soil quality and fertility. In vineyard contexts, soil microbiology is now considered to be one of the key factors influencing the health of the vines and the quality of the grapes. Exploring how soil microbial communities are related to soil ecosystem functioning is therefore becoming an essential issue for soil conservation strategies. Integrating information concerning LULC history is strongly recommended in the quest to evaluate and adopt the best strategies for developing sustainable management practices.

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