

Vineyard renewal reduces copper and zinc bioavailability and increases microbial diversity in southern Brazil

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Abstract

The long-term use of vineyards and their renewal causes numerous chemical changes in the bioavailable copper (Cu), zinc (Zn) levels, organic carbon and microbial properties of the soil. However, there are few works with an approach to the use of vineyards in subtropical soils. Thus, the objective of this work was to evaluate the relationship between physical-chemical properties, microbial basal respiration, activity, richness, and diversity from vineyards and nearby forest reference soils in three different areas in southern Brazil. Each area underwent three treatments: old cultivation, i.e., a well-established vineyard; renewed vineyard; and reference, a native forest located adjacent to the vineyards. The physical-chemical properties clay content, moisture, pH, organic matter (OM), carbon (C), nitrogen (N), phosphorus (P), potassium (K), copper (Cu), and zinc (Zn) level; the basal respiration (BR), microbial biomass Carbon (MBC), microbial biomass Nitrogen (MBN), hydrolysis of fluorescein diacetate (FDA), microbial quotient (qMic), and metabolic quotient (qCO₂); and microbial richness and Shannon diversity index were determined. The organic C level differently influenced the microbial activity and richness in each of the three experimental areas. The change in microbial diversity indexes impacted soil biological activity in two locations. Vineyard renewal promotes the reduction of Cu (- 48.8%) and zinc (- 20%) bioavailability and increases microbial diversity in subtropical soils in southern Brazil.

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ABSTRACT

The long-term use of vineyards and their renewal causes numerous chemical changes in the bioavailable copper (Cu), zinc (Zn) levels, organic carbon and microbial properties of the soil. However, there are few works with an approach to the use of vineyards in subtropical soils. Thus, the objective of this work was to evaluate the relationship between physical-chemical properties, microbial basal respiration, activity, richness, and diversity from vineyards and nearby forest reference soils in three different areas in southern Brazil. Each area underwent three treatments: old cultivation, i.e., a well-established vineyard; renewed vineyard; and reference, a native forest located adjacent to the vineyards. The physical-chemical properties clay content, moisture, pH, organic matter (OM), carbon (C), nitrogen (N), phosphorus (P), potassium (K), copper (Cu), and zinc (Zn) level; the basal respiration (BR), microbial biomass Carbon (MBC), microbial biomass Nitrogen (MBN), hydrolysis of fluorescein diacetate (FDA), microbial quotient (qMic), and metabolic quotient (qCO₂); and microbial richness and Shannon diversity index were determined. The organic C level differently influenced the microbial activity and richness in each of the three experimental areas. The change in microbial diversity indexes impacted soil biological activity in two locations. Vineyard renewal promotes the reduction of Cu (- 48.8%) and zinc (- 20%) bioavailability and increases microbial diversity in subtropical soils in southern Brazil.

Keywords: bacterial diversity, microbial activity, soil management, soil vineyard, *Vitis* sp.

Introduction

Wine-growing in the southern Brazil started over 100 years ago. Nowadays, the viticulture region is the largest in Brazil with about 32 thousand hectares of vineyards under cultivation (Protas and Camargo, 2011). Nevertheless, old vineyards, with more than 50 years, have presented high soil sub-surface acidity, nutrient imbalance, and pathogenic fungi contamination which, in general, reduce the productivity and quality of the grapes. The most common problems are related to soil biotic factors, such as the presence of harmful insects and fungi, and abiotic factors, such as low pH, nutritional imbalance, presence of phytotoxins, highly clayey soils, and contamination by metals (Garrido et al., 2004; Schneider et al., 1999).

Among metal pollutants in vineyard soils, Cu is the most common contaminant. Cu has been used as a fungicide and bactericide in agricultural production systems for many years (Melo et al., 2013; Pavlovic, 2011). The constant spraying of Cu-containing pesticides in vineyards has led to Cu accumulation, in the superficial layers of the soil. Such problem is aggravated by the low pH of soils, low cation-exchange capacity, and low organic matter (OM) level. Thus, Cu becomes mobile and potentially bioavailable in the soil, causing toxic effects to plants and beneficial microorganisms (Bruins et al., 2000; Brunetto et al., 2014; Komárek et al., 2010; Mackie et al., 2012; Pavlovic, 2011).

Additionally, some studies report that Cu contamination in vineyard soils induces changes in microbial communities in the superficial layers, affecting structural diversity and shifting the resistance of microorganisms to this metal (Dell'Amico et al., 2008; Lejon et al., 2010). Several studies showed that soils with long-term Cu pollution is determining factor that affect the soil microbial community (Fernández-Calviño et al., 2010; Hansda et al., 2017; Mackie et al., 2013). Within this context, there is a large gap of information about the microbial community associated with vineyard soils under long cultivation.

Soil microorganisms are responsible for many functions including nutrient cycling and the degradation of xenobiotics (Baath, 1989; Giller et al., 1998). High concentrations of metals in soil cause transient effects on microbial communities (Azarbad et al., 2016; Nannipieri et al., 2003). These effects can be assessed by the measurements of microbial activity, enzymatic activity, basal respiration and microbial biomass. Such attributes are often used as indicators of soil environmental changes (Anderson, 2003; Schlöter et al., 2003). In viticulture, soil indicators such as microbial biomass carbon (MBC), microbial quotient (qMic), and metabolic quotient (qCO₂) are reported as being sensitive to changes resulting from management practices (Cavani et al., 2016). Coupled with these biochemical techniques, new approaches based on DNA sequencing,

mainly from conserved regions of 16S rRNA subunit have allowed researchers to demonstrate more precisely microbial community abundance at the taxon level and how the microbes are affected by environmental factors and human activities (D'Amore et al., 2016; Gilbert et al., 2014; Hermans et al., 2016; Nielsen et al., 2015; Oulas et al., 2015). The changes caused by the renovation of vineyards is still little explored in relation to the impact on the bioavailability of elements found in large quantities such as Cu and Zn (Andreazza et al., 2010). In addition, there is an important information gap in relation to the effects of renewal such as the contribution of organic compounds to the soil and its oxidation by soil preparation operations and the impact on soil bacterial diversity.

The low soil pH and Cu toxicity commonly found in long term vineyards cultivation, are associated with changes in soil microbial community and activity, we hypothesized that increasing soil pH and as decreasing Cu bioavailability thought vineyard renovation, would also potentialize microbial community changes increasing microbial diversity and activity. To test this hypothesis, we evaluated the relationship between soil physical-chemical properties, microbial activity, richness and diversity of the soils from old and renewed vineyards in southern Brazil. Native pristine areas were also used withing this work as a reference.

Materials and Methods

1. *Description of the areas and experimental design*

The experimental areas were located in a traditional winery region in southern Brazil (Figure 1). Three different cities were chosen stablishing the experiment: Caxias do Sul (CX), Flores da Cunha (FC), and Farroupilha (FA). For each city, three treatments were applied as follows: old vineyard, renewed vineyard, and reference native area. The treatments under old cultivation were located in a portion of a well-established vineyard. The old vineyards of Caxias do Sul (CX), Flores da Chunha (FC) and Farroupilha (FA) had 100, 15 and 15 years of establishment, respectively. The treatments under renewed cultivation were located in a section of an old vineyard that was managed to recover the physical-chemical conditions before establishing new vines. The treatments under a native area were located adjacent to the vineyards and were used as a reference.

Each treatment was set in an area of 675 m². The precise location and descriptions of the areas are listed in Table 1. The old vineyards were all cultivated using the trellis system, and the renewed vineyards used the variety Paulsen 1103 as rootstock. The soils in the region were classified as Lithic Udorthent (IUSS Working Group WRB, 2015). The vineyard renewal treatments consisted of removing the old grapevines, preparing the soil with plowing and harrowing, application of organic compost (5 Mg ha⁻¹) and establishment of oat and white clover as a cover crops between the vines. The addition of organic compost and the use of cover crops are recommended best practices for the renewal of vineyards in Brazil. In addition, in the renovation of the vineyard, the soil is prepared with plowing and harrowing for physical improvement of the soil.

Soil sampling

In the CX area, six soil samples (0-0.10 m depth) were collected from six different spots of each treatment, i.e., three within the rows of the vines and three between the rows of vines. Additional three soil samples were collected from the native treatment. A total of fifteen samples were obtained from the CX area. In the FC and FA areas, three soil samples were collected from three different spots of each treatment, all three within vine rows, for a total of nine samples per area. In total, the soil database was composed of 33 soil samples.

For the bacterial community analysis, disposable spatulas were used, and the soil samples were placed in sterile 15 ml plastic tubes. At the same time and spot, a soil sample of approximately 1 kg was collected using an auger for the soil properties analysis. All the soil samples were collected in July 2016 when the vines were in the dormant stage, and the samples were immediately stored at 4 °C until analysis.

Soil Physical-chemical Properties

The soil moisture content was determined by drying the soil in an oven at 105 °C for 24 hours and measuring the difference between the wet and dry sample weights. For the physical-chemical properties analysis, the soil samples were dried at 45 °C until the weights did not vary more than 0.01% and sieved using a 2 mm mesh. The clay content was determined with a densimeter (Bouyoucos, 1962). The concentration of H⁺ (pH) in the soil was measured with a pH meter (Geaka model PG2000) in 1:1 soil to water suspension. The organic matter (OM) and carbon (C) level were assessed following the Walkley–Black method (Walkley and Black, 1934). For the measurement of the nitrogen (N) level we used the Kjeldahl method (Bremner, 1960). For phosphorus (P), potassium (K), copper (Cu), and zinc (Zn) level determination, the soil samples were subjected to Mehlich-1 extraction, and the soil extracts were analyzed using inductively coupled plasma–optical emission spectrometry (ICP-OES) (Perkin Elmer, Inc., Waltham, Massachusetts, USA) following the standard operational procedures. All the determinations were done in triplicate, and the results were expressed on a dry weight basis.

Measurement of microbial biomass, basal respiration and enzyme activity

The soil samples were homogenized and sieved using a 2-mm sterile mesh. The basal respiration (BR) was measured following the Norman and Peech (1965) method (Norman and Peech, 1965). The microbial biomass carbon (MBC) and nitrogen (N_{mic}) were quantified by irradiation-extraction following the methodology proposed by Monz et al. (1991). The soil samples were heated in a microwave oven (Model NN-560BH, Panasonic, Newark, NJ, USA) at 120 v (60 Hz), 2,450 MHz, and 2.52 x 10⁵ J for 3 min. The MBC and the MBN were extracted with a 0.5 M K₂SO₄ solution and determined by wet combustion and Kjeldahl digestion, respectively.

The total soil microbial activity was determined using the fluorescein diacetate (FDA) hydrolysis method (Schumacher et al., 2015). The microbial quotient (q_{Mic}) was obtained by the ratio of the total organic C (using the method described above) and MBC (Anderson and Domsch, 1978; Gama-Rodrigues and Gama-Rodrigues, 2008). The metabolic quotient (qCO₂) was calculated by the ratio between BR and soil microbial biomass per unit time (Anderson and Domsch, 2010). It expresses how much CO₂ is released by microbial biomass as a function of time, representing the specific respiration rate of the sampled microbial biomass (Alves et al., 2011). To obtain qCO₂, the BR of the soil samples was measured for 30 days, with the BR result as a sum of those 30 days. All the determinations were done in triplicate, and the results were expressed on a dry weight basis.

Physical-chemical properties and microbial indicators data were analyzed by ANOVA, and, when the results were significant (p ≤ 0.05), the means of the parameters were compared using the Tukey test (α = 0.05) using the *Agricolae* package (Mendiburu, 2019). Finally, Pearson’s correlation coefficients were calculated using the “Hmisc” package (Harrell and Dunpont, 2019) to investigate the relationship between the physical-chemical properties, microbial indicators, and the bacterial diversity measurements of soil samples. Simple linear correlations were computed using Microsoft Excel.

Soil microbial Richness and Diversity Analysis

Microbial richness and diversity were performed by sequencing of the 16S rRNA gene as described previously (Carlos et al., 2021). The fastq files were analyzed following the recommendations of the Brazilian Microbiome Project (Pylro et al., 2014). Downstream analyses were carried out using a rarefied dataset of sequences as previously recommended by Lemos et al. (2011). Data analysis was performed into the R environment (R Development Core Team, 2008) using the phyloseq package (McMurdie et al., 2013) for the estimation of bacterial diversity and richness.

Results

1. Chemical Properties of the Soil

Was observed that the average levels of Cu in the old areas were 167 mg dm⁻³ and in the renewed vineyards 85 mg dm⁻³. Thus, the renewal of vineyards contributes to a significant reduction (- 48.8%) of bioavailable

Cu levels in the soil. Soil Cu levels are higher in areas with old vineyards and where grapes have been cultivated for a longer time, such as in CX. In general, there is a large hyper accumulation of Cu from the old vineyards (+ 5.080%) and from the renewed vineyards (+ 3.160%) in relation to native areas where there was no grape cultivation (Figure 2). With the renewal of the vineyards, a significant reduction (- 20%) of the bioavailable Zn contents in the soil was also observed. The mean bioavailable levels of Zn from the three sites in the old vineyards were 33 mg dm⁻³ and in the renewed vineyards 23 mg dm⁻³. An increase of 230% and 160% was observed in the levels of Zn in the old and renewed vineyards in comparison to the native areas, respectively (Figure 2). Vineyard renewal did not affect soil organic carbon content (Figure 2). A reduction in soil organic carbon level was observed in old (- 26%) and renewed (-33%) vineyards in relation to native areas (Figure 2). The total N content of the soil also had a reduction of 22.8% of the renovated vineyards in relation to the old vineyards (Figure 2). In relation to native area, total soil nitrogen had lower levels in old (- 55%) and renewed (- 72%) vineyards (Figure 2). Phosphorus levels increased in old vineyards (+ 381%) and renewed vineyards (+ 296%) in relation to the native area (Figure 2). Potassium levels were not significantly different in relation to the types of vineyard (Figure 2).

Microbial Indicators of the Soil

Basal respiration had a reduction (- 37%) in renewed vineyards compared to old vineyards (Figure 3). In CX and FA there was lower basal respiration in the old and renewed vineyards compared to the native area. The MBC also had a reduction of 15.7% due to the renovation and the MBN a 38.1% increase in the renewed vineyards in relation to the old vineyards (Figure 3). MBN levels were generally lower in old vineyards compared to native areas. FDA activity was little affected in relation to vineyard renewal. The FDA activity had a reduction in soil activity was observed in old vineyards (- 31%) and renewed (- 51%) in relation to native areas (Figure 3).

The qMic parameter is a measure of the amount of living biomass present in the soil's OM (Table 2). There was a significant difference ($p < 0.05$) in qMic only in the soil samples collected in the CX area, and the soil samples from the native and old treatments displayed lower qMic values in comparison to the renewed and old vineyards. In the FC and FA areas, there was no significant difference between treatments, but the qMic value was lower in the soil samples collected from the native and renewed treatments in the FC area and from the renewed treatment in the FA area.

The qCO₂ parameter expresses the respiration rate per unit of microbial biomass per day and becomes lower via the loss of C-qCO₂ by the BR (Table 2). Thus, the greater the efficiency in using C, the lower the qCO₂ value. In all three areas, qCO₂ differed significantly among treatments; the parameter was lower in the soil samples collected from the native treatment in the CX and FC areas and, in the FA area, was lower for the renewed treatment. The qCO₂ values were lower in the old and native treatments in the CX area, indicating that C metabolism of the soil samples collected from old vineyards and native area in the CX is more efficient.

3.3 Archaeal and Bacterial Richness and Diversity

From the analysis of the partial sequencing of the 16S rRNA region, a total of 1,161,315 high-quality sequences were obtained after filtering by quality. On average 35,191 sequences were obtained per sample (average length of 200 bases and error approaching 0.5). The coverage analysis indicated that 93–95% of all OTUs in the soil samples were captured. Boxplots illustrating the variation of the microbial alpha diversity measurements of the soil samples gathered from the different treatments in the studied areas are displayed in Figure 4.

In the CX area (Figure 4), the old treatment presented the highest microbial observed richness (2526.67) and Shannon index (6.8894), while the native treatment showed the lowest (2205.33 and 6.4844, respectively). In CX, higher shannon index values were observed in the renewed vineyard compared to the old vineyard. In the FC area (Figure 4), the highest values for observed richness (2547.33) and Shannon Index (6.9507) were found in the soil samples collected from the old treatment, and the soil samples collected from the renewed treatment displayed the lowest (1814.33 and 6.1942, respectively). As shown in the ANOVA, the observed

richness ($p = 0.0006$) and the Shannon index ($p = 0.0001$) differed significantly depending on the treatments in this area. The microbial observed richness ($p=0.0221$) and Shannon index ($p=0.0217$) of the soil samples collected from the different treatments differed significantly in the FA area (Figure 4). The observed richness and Shannon were higher for the soil samples from renewed treatments (2748.33 and 6.995, respectively) and lower for the soil samples from old treatment (2174.33 and 6.6051, respectively) in this area.

3.4 The relationship between physical-chemical properties, microbial parameters and indicators, and bacterial alpha diversity measurements of the soil

Pearson's correlations were calculated to understand the relationships between the physical-chemical properties, the microbial parameters and indicators, and microbial observed richness and Shannon diversity index of the soil samples collected from old and renewed vineyards. Simple linear regressions of the correlations were also calculated to determine the percentage of the variation of the analyzed variables. The Pearson's correlation of each studied area is illustrated in Figure 5.

For the CX area (Figure 5), there was a positive and significant relationship between most of the microbial parameters and indicators analyzed in the present research. The relationship between Cu level with BR and Cu with MBC were negative and significant, but both displayed a moderate correlation ($0.5 < r < 0.7$). The simple linear regression of the correlation between Cu level and RM ($y = -1.7369x + 992.87$, $R^2 = 0.3187$) demonstrated that 31.8% of the BR variation can be explained by the Cu level of the soil in the CX area. The correlation of soil enzymatic activity, as measured through FDA hydrolysis, with the Cu level was negative but not statistically significant.

Correlations between the Cu level and the alpha diversity index measurements were statistically significant and positive, with an $r=0.73$ for the observed richness and $r=0.61$ for the Shannon index (Figure 4). These positive correlations may be an indication that the soil microbiota has managed to adapt to the stressed condition of high level of Cu over the years. The simple linear regression calculated for the relation between soil Cu level and observed richness ($y = 1.1207x + 2183.8$, $R^2 = 0.535$) demonstrated that the variation in Cu level can explain 53.3% of the observed richness. For the Shannon index and the soil Cu level correlation, the simple linear regression ($y = 0.0012x + 6.5141$, $R^2 = 0.3681$) demonstrated that the variation on the Cu level can explain 36.8% of the Shannon index. MBC and BR were both positively correlated with the soil total C level moderately ($r=0.62$) and strongly ($r=0.91$), respectively, and MBC was strongly positively correlated to the soil N level ($r=0.87$) in the CX area.

In the FC area (Figure 5), the BR was strongly and positively correlated to the qCO_2 ($r = 1$) and the observed richness ($r = 0.72$). The enzymatic activity measured by the FDA hydrolysis decreased (Figure 5) meanwhile the Cu concentrations increased in the analyzed soil samples, and this negatively and strongly correlated relationship was demonstrated in the Pearson correlation values between Cu level and FDA hydrolysis ($r = -0.8$). The decrease in this microbial activity indicator may demonstrate that certain concentrations of Cu have a suppressive effect on the metabolic functions of microbial cells. A strong and negative correlation was also observed between Cu level and the Shannon diversity index ($r = -0.76$) for this area.

The Pearson's correlation for the FA area is illustrated in Figure 5, and just a few variables were correlated. The relationship between BR and qCO_2 was positive and very strong ($r = 0.98$), and the simple linear regression ($y = -0.0269x + 9.2344$, $R^2 = 0.684$) demonstrated that the increase in the Cu level caused a decrease in the FDA hydrolysis in the soil samples, corresponding to 68.4% of the variation. Among the alpha diversity indexes, only the observed richness displayed a significant and negative correlation with the Cu level ($r = -0.67$), and the simple linear regression ($y = -2.6606x + 2560.9$, $R^2 = 0.4487$) showed that the Cu level corresponded to 44.8% in the variation of observed richness of the soil samples collected from the FA area.

In the analysis of the principal components (PCA) it was observed that the vectors of Cu and Zn were more directed to the groups of the old and renewed vineyards in the same direction as the vector of the shannon diversity index (Figure 6). The metabolic quotient vector, on the other hand, is directed towards the grouping of old vineyards. On the other hand, the vectors directed to the grouping of the reference area

(native) were the MBC and N vectors (Figure 6).

Discussion

In the present study, a combination of physical-chemical, biological, molecular, and statistical analyses was applied to understand whether the microbial communities in the soils from old and renewed vineyards differed. The results demonstrated that the soil management used for the renovation of the vineyards caused changes in the microbial community in the studied areas in terms of the microbial parameters and indicators and bacterial diversity indexes. Our results are in agreement with other studies that demonstrated that microbial parameters and bacterial indexes change according to soil management practices and contamination levels. It provides important information on how the soil is responding to these new conditions (Anderson and Domsch, 1993; Gama-Rodrigues and Gama-Rodrigues, 2008; Giller et al., 1998).

The reduction of bioavailable Cu and Zn occurred due to vineyard renewal. Possibly the addition of organic compost to the soil contributed to the increase of organic molecules that form more stable bonds, complexation, with Cu and Zn and reduce their bioavailability (Andreazza et al., 2010). In addition, the more intensive use of cover crops, such as oat and white clover, also enhances the phytostabilization of Cu and Zn and contributes to the reduction of these elements (Andreazza et al., 2010). According to the initial hypothesis of the study, the reduction of Cu and Zn contents in the soil increases the diversity of soil microorganisms. In the current study, high levels of bioavailable Cu were found in old CX and FC vineyards. In these two locations, an increase in the shannon index was observed with the renewal of the vineyard, concomitant with a reduction in the levels of Cu and Zn in the soil. On the other hand, in FA, where the Cu content in the old vineyard was low, there was no increase in the shannon index with the renewal of the vineyard. Other soil factors may interfere with the ecology of soil microbial interaction. In CX, the increase in soil carbon and nitrogen content reduced the Shannon index. Other authors have also observed that increasing soil organic carbon as a source of energy and nutrients such as nitrogen benefits some specific microorganisms that have greater dominance compared to others and there is a reduction in soil microbial diversity (Carlos et al., 2020).

The BR is a method of measuring the metabolic activity of the soil microbiome; however, it should be performed and interpreted with caution. High respiratory activity can result by both, the availability of labile carbon substrates, where the decomposition of organic matter is intense, and from the rapid oxidation of substrate due to the influence of some environmental factor (Tótola and Chaer, 2002). Additionally, the BR has generally a significant and strong correlation with soil organic C level and also with most of the microbial parameters of the soil (Anderson and Domsch, 2010; Norman and Stotzky, 1965); however, only for the CX area were the correlations between the C and BR and the C and MBC was significant and positive.

The significant correlations between MBC level and BR with organic C level in the soil samples indicate that higher levels of organic C may benefit soil microbial biomass and enzymatic activities. The soil microbial biomass acts as a nutrient reservoir that is gradually released into the soil via microorganism death (Singh, 2015).

Organic carbon is an energy source for the microbial community, moreover, due to its chelating effect, protects soil microorganisms against excessive levels of metals (Balota, 2017; Lejon et al., 2010). The C level, as well as the pH, can influence FDA hydrolysis enzymatic activity, which here showed significant differences between the treatments of the three studied areas and displayed higher values in the soil samples collected from reference treatments (Figure 3). The increase in the enzymatic activity of the FDA hydrolysis is due to the addition of residues to the soil since this enzyme actively participates in the soil organic matter transformation (Lopes et al., 2010).

FDA activity decreases due to high Cu levels in soil contaminated by this metal (Arthur et al., 2012; D'Ascoli et al., 2006). This decrease is a piece of evidence that high levels of Cu in the soil influence microbial activity negatively, since the decrease in the FDA hydrolysis indicates a reduction of microbial activity (Adam and

Duncan, 2001). This negative correlation between Cu level and FDA hydrolysis in the soil was found in FC and FA areas (Figure 5).

High qCO_2 values mean that the microbial population is oxidizing carbon from their cells (maintenance respiration of living microorganisms), so the microbiome is under a stressed condition (Anderson and Domsch, 2010; Islam and Weil, 2000). Generally, in a heavy metal-contaminated ecosystem, such as those with high levels of Cu, higher values of qCO_2 are expected in comparison to a natural ecosystem (Anderson and Domsch, 2010). Nevertheless, Cavani et al. (2016) did not observe differences in qCO_2 values in soil samples collected from contaminated vineyards with diverse levels of Cu.

The qCO_2 value tends to decrease in stable environments, which probably happened in the soil from native and old treatments in the CX area. In addition, soil management practices, such as vegetation cover replacement and soil tilling before vine replanting, can accelerate the organic matter decomposition, and, as a consequence, cause a qCO_2 increase (Balota, 2017). This effect was observed in the soil samples gathered from the RN vineyards. In RN vineyards of the CX and FC areas, the soil was worked to incorporate organic fertilizer and correct the fertility, and the qCO_2 was higher in RN treatments (Table 2). Meanwhile, a different behavior was observed in the FA area. The soil sample collected from native treatment displayed a higher value of qCO_2 in comparison to other treatments, and this may be related to the fact that the reference of this area is a field with a predominant grass cover.

The observed richness and Shannon diversity index that were used to measure the microbial diversity in the soil samples differed among treatments in the FC and FA areas. The changes caused by management practices directly impact the diversity and composition of soil microbial communities, and the relationship between microbial diversity and disturbed habitats is very complex. Besides, this relationship is also dependent on the disturbance degree; some disturbed habitats may have higher diversity than natural environments for example (Montecchia et al., 2015; Szoboszlay et al., 2017).

The microbial diversity did not differ in the soil samples collected from an area with a history of Cu contamination in Denmark (BERG et al., 2012). Our results for the CX area are similar since the observed richness and Shannon diversity index did not differ among the treatments in this area. In contrast, other studies reported that bacterial diversity was influenced negatively by the presence of high concentrations of Cu in soils cultivated with fruit trees (Dell'Amico et al., 2008; Powell et al., 2011). For the FA area, our results agree with those studies, as the observed richness and Shannon diversity index were lower in the soil samples from the AL vineyards where Cu levels were higher in comparison to the RN and native treatments.

OM level has also an effect on soil microbial diversity. Soil Cu availability is related to the OM level among other factors, and the available Cu level is capable to influence the bacterial diversity of soils poor in OM (Fernández-Calviño et al., 2010; Ippolito et al., 2010; Wakelin et al., 2014).

Conclusion

Vineyard renewal management practices reduce copper and zinc bioavailability and increases microbial diversity in subtropical soils in southern Brazil. Vineyard renewal promotes a reduction in microbial biomass carbon and soil basal respiration. Higher level of organic carbon and total soil nitrogen reduce the shannon diversity index in soils cultivated with vineyards in southern Brazil. The long-term adoption (~100 years) vineyard cultivation increases bioavailable levels of copper and zinc in soil by up to 5,080 and 230%, respectively. The high level of copper to zinc in the soil cultivated by vineyard for long term increases stress conditions in the microbial biomass indicated by the high value of the metabolic quotient.

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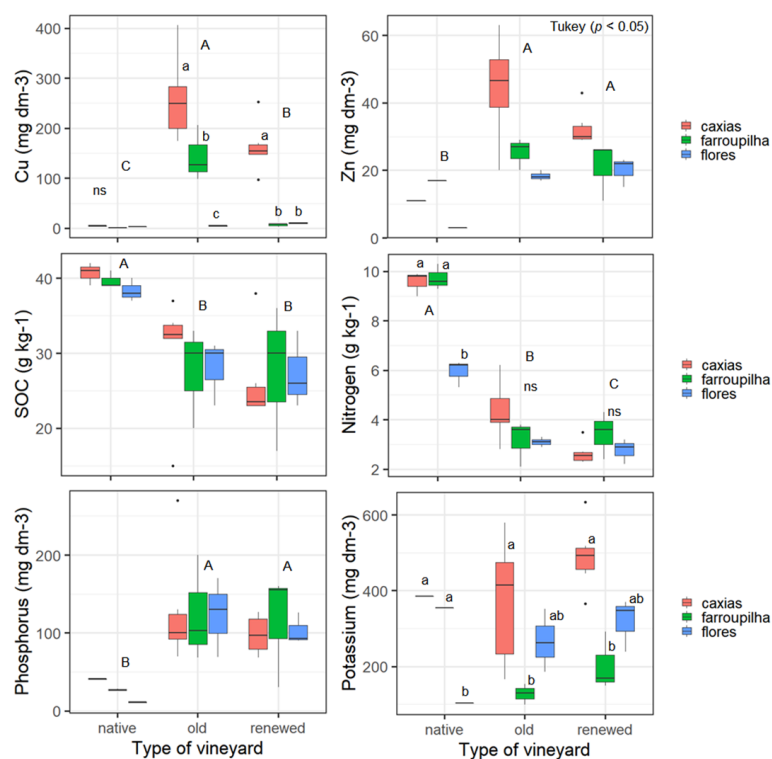
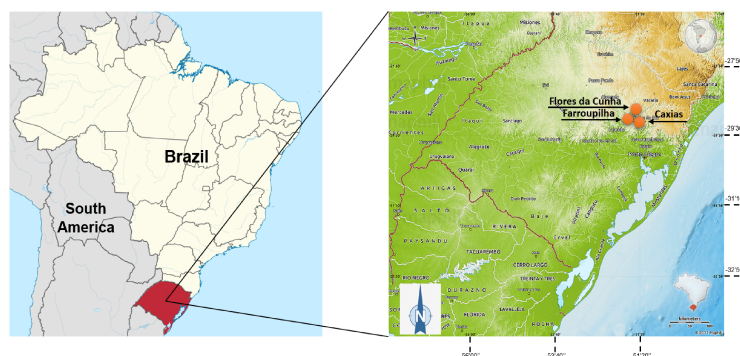
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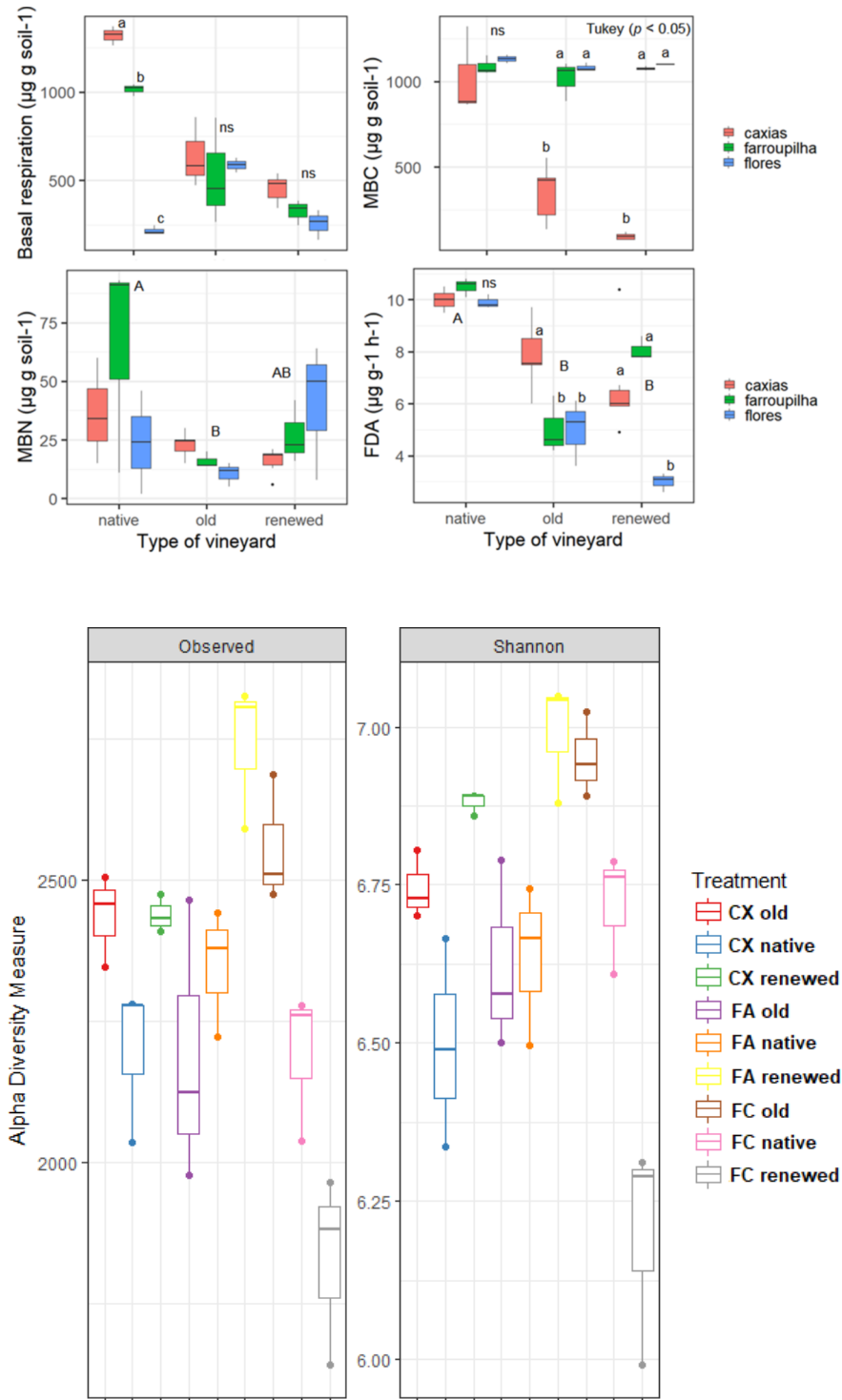
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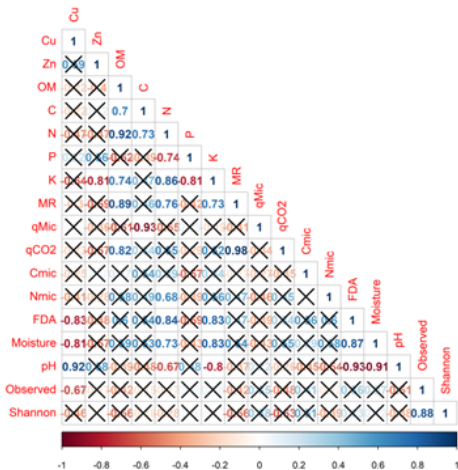
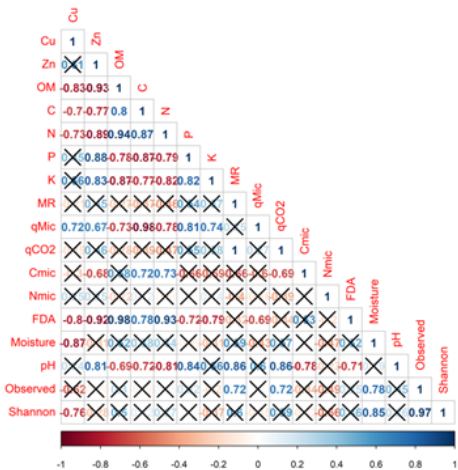
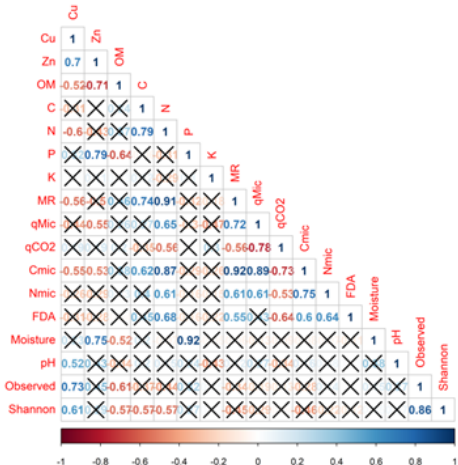
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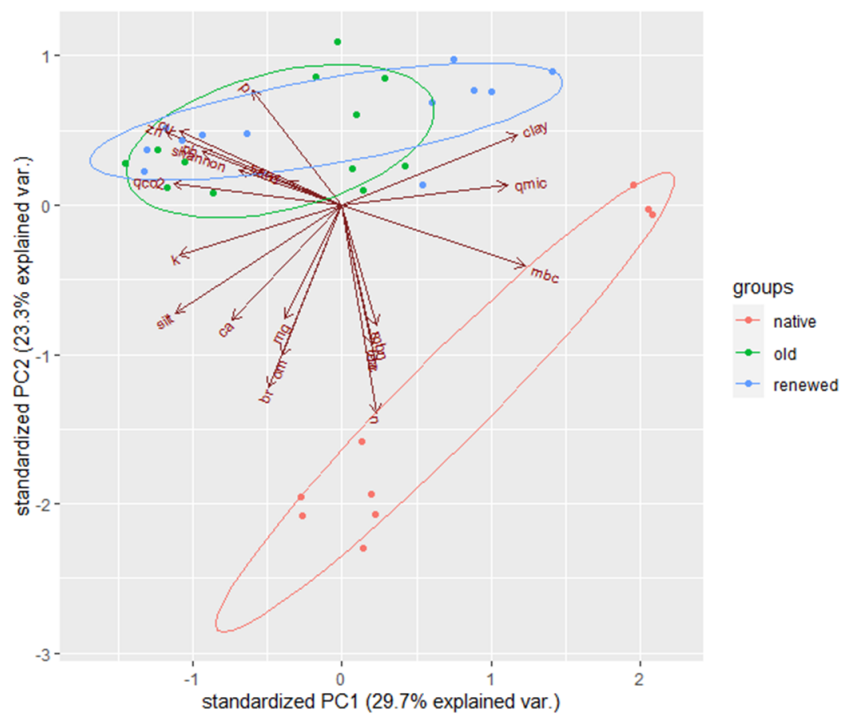
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