Antonie van Leeuwenhoek (2015) 107:935–949
DOI 10.1007/s10482-015-0386-6

ORIGINAL PAPER

Microbiological functioning, diversity, and structure of bacterial communities in ultramafic soils from a tropical savanna

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Received: 15 November 2014 / Accepted: 13 January 2015 / Published online: 24 January 2015

Abstract Ultramafic soils are characterized by high levels of metals, and have been studied because of their geochemistry and its relation to their biological component. This study evaluated soil microbiological functioning (SMF), richness, diversity, and structure of bacterial communities from two ultramafic soils and from a non-ultramafic soil in the Brazilian Cerrado, a tropical savanna. SMF was represented according to simultaneous analysis of microbial biomass C (MBC) and activities of the enzymes β-glucosidase, acid phosphomonoesterase and arylsulfatase, linked to the C, P and S cycles. Bacterial community diversity and structure were studied by sequencing of 16S rRNA gene clone libraries. MBC and enzyme activities were not affected by high Ni contents. Changes in SMF were more related to the organic matter content of soils (SOM) than to their available Ni. Phylogeny-based methods detected qualitative and quantitative differences in pairwise comparisons of bacterial community structures of the three sites. However, no correlations between community structure differences and SOM or SMF were detected. We believe this work presents benchmark information on SMF, diversity, and structure of bacterial communities for a unique type of environment within the Cerrado biome.

Keywords Cerrado · Metals · Soil enzymes · Microbial biomass carbon · 16S rRNA gene

Introduction

Ultramafic soils, present in less than 1 % of the Earth's surface (Kazakou et al. 2008), are covered by a unique endemic and highly adapted flora. They originate by the weathering of different types of ultramafic rocks, and are characterized by high levels of metals such as Ni, Co and Cr, low levels of N, P, K and Ca, and a high Mg/Ca ratio (Brooks 1987). These soils have been studied for their geochemistry and its relation to their biological component, their plant communities, and the occurrence of physiological mechanisms related to metal hyperaccumulation in plants (Reeves et al. 2007).

In Brazil, ultramafic areas of the state of Goiás and Tocantins are economically important due to the intensive mining started in the 1970s. They contain

Electronic supplementary material The online version of this article (doi:10.1007/s10482-015-0386-6) contains supplementary material, which is available to authorized users.

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over 100 million tons of extractable nickel ore, which represents 2 million tons of metallic Ni (Reeves et al. 2007). These operations have resulted in degradation of extensive areas covered with natural vegetation, and thus studying their soils and their plant communities is necessary to prevent further damage to these ecosystems (Reeves et al. 2007). Due to their unique geochemical properties, ultramafic soils offer the opportunity to study microbial communities that have been exposed to high metal concentrations since their formation (Schipper and Lee 2004). Additionally, microbial communities of the rhizosphere soil have been affected by an adapted endemic flora. Therefore, these microbial communities probably present strong evolutionary and physiological responses to their local environment, and are as specialized as the endemic plant communities growing in these areas (Oline 2006). Some research groups have begun investigating biotechnological applications of ultramafic soil bacteria, for both metal bioremediation and phytoextraction (Rajkumar et al. 2009; Prasad et al. 2010). For example, nickel mobilizing, plant-growth promoting bacteria have been isolated and tested for their potential in promoting plant growth and Ni accumulation in *Brassica* (Ma et al. 2009a, b).

Metal contamination may reduce total microbial biomass (Brookes and Mcgrath 1984; Fliessbach et al. 1994; Wang et al. 2007), change microbial community structure (Frostegard et al. 1996; Griffiths et al. 1997; Sandaa et al. 1999; Kandeler et al. 2000; Mengoni et al. 2001), and affect enzyme activities (Kandeler et al. 2000; Wang et al. 2007), or physiological indices such as the metabolic quotient (qCO_2) (Renella et al. 2007). However, indirect effects caused by limited metal bioavailability, or developed resistance to heavy metal pollution (Nannipieri et al. 2012) may lead to contrasting results, such as those reported by Niklinska et al. (2006), who found no differences between Zn and Cu polluted and unpolluted sites regarding biomass, activity and physiological profiles of microbial communities. Renella et al. (2004), for instance, found that high Cd-contaminated soils showed low amounts of bioavailable Cd, and although bacterial community structure was not affected by these low Cd concentrations, enzyme activities and respiration differed between samples.

It can be hypothesized that microbial communities of ultramafic soils may be negatively influenced by their typically high metal concentrations. However, Schipper and Lee (2004) found that increasing metal concentrations in six ultramafic soils were not correlated with biological properties such as the catabolic response profile, microbial biomass and respiration, and qCO2. Oline (2006) used 16S rRNA gene sequencing and showed that ultramafic bacterial communities were more similar to each other than to non-ultramafic communities. Other studies using 16S rRNA gene sequencing of bacterial communities in ultramafic environments included analyses of natural versus disturbed and revegetated sites (Herrera et al. 2007), and the rhizosphere of Ni hyperaccumulators (Idris et al. 2004).

Although studies on plant communities from the ultramafic complex of Barro Alto have been carried out (Reeves et al. 2007), nothing is known about the composition, structure, and activity of microbial communities from these areas. These communities are an important component of ultramafic environments due to their role in nutrient cycling. Therefore, knowledge on the diversity, structure, size and activity of the microbial communities of ultramafic soils in the Cerrados may help to understand the microbiological functioning of these soils and give suggestions on indicators, which will also be used for assessing soil quality in soils under restoration. Microbial biomass, soil enzyme activities and respiratory rates are considered robust microbiological indicators of soil quality and may serve as indicators of land-use change (Doran and Zeiss 2000; Yao et al. 2000; Waldrop et al. 2000; Dawson and Smith 2007).

In the same way that metal pollution affects microbial communities in contaminated areas, we expected that higher quantities of available Ni of undisturbed ultramafic soils would affect biomass, activity, composition, and structure of soil microbial communities, influencing soil functioning. Thus, the main goal of this study was to evaluate microbial biomass C, soil enzyme activities related to C, P and S cycles, and richness, diversity, and structure of bacterial communities of ultramafic soils of Central Brazil.

Materials and methods

Soils and sampling

Sites of the ultramafic complex of Barro Alto, in the Brazilian state of Goiás were first selected based on

topographical, geochemical and geological observations (Geology Department of Anglo American). Chemical properties of these sites (pH, organic matter contents, Ca^{2+}, Mg^{2+}, Al^{3+}, K^{+}, P, and DTPA-extractable metals, including Ni), as well as their floristic composition, richness and abundance of plant species, and plant Ni content were determined (Andrade et al. 2011; Aquino et al. 2011a, b). Based on these data, soil samples were collected from two areas; soils from Site 1, an area named SAP (15°06′04.4″S; 49°00′38.4″W) were loamy Cambisols mainly composed of saprolites, while soils from Site 2, named LAT (15°06′31.1″ S; 49°01′15.0″ W), were sandy clay loam Oxisols mainly composed of laterites. Site 1 presents a "Campo Sujo" physiognomy, which is defined as a grassland formation with grasses and small and sparse shrubs (Ribeiro and Walter 1998; Oliveira-Filho and Ratter 2002) (online resource 1A). Site 2 presents a "Cerrado Ralo" physiognomy, a savanna with small trees whose trunks are characteristically twisted, mixed with shrubs and an herbaceous layer (Ribeiro and Walter 1998; Oliveira-Filho and Ratter 2002) (online resource 1B). An adjoining non-ultramafic Cerrado soil sample (15°05′05.0″S; 48°58′54.6″W), with a "Cerradão" physiognomy (a forest formation with 50–90 % tree coverage) was also collected. The type of soil in this area was a clay loam Oxisoil. Sites 1 and 2 are 1.37 km apart from each other. The Cerrado site is located 3.6 km from Site 1 and and 4.97 km from Site 2. Plant species composition differed between lateritic and saprolitic areas (Aquino et al. 2011a, b; Andrade et al. 2011). Local climate is Cwa (according to the Köppen classification).

In each area, we defined three replicate plots along a transect line. Total sizes of the areas varied between 30 m × 10 m and 60 m × 10 m. In each plot, ten soil samples were collected to form a composite sample. Litter layer was removed before soil sampling. Soil was collected from the 0 to 10 cm layer with a sterilized soil probe, and mixed in two sets of plastic bags: samples for microbial biomass C and enzyme activity measurements were kept on ice before transport to the laboratory the same day, where they were stored at 4 °C and analyzed the same week; samples for DNA extraction were kept on dry ice and stored in the laboratory at −20 °C. Samples were sieved through a 4-mm mesh, in order to remove plant debris and roots. Soil samples were collected in December 2009. During this month the mean precipitation value in Barro Alto (1996–2010) is 219.39 mm.

Soil chemical analyses

Chemical properties of soil samples were measured using routine methods: soil pH was measured at a soil:water ratio of 1:2.5 (w/v) (Jackson 1958); P and K were extracted with Mehlich-1 solution (0.0125 M H_2SO_4 + 0.05 M HCl) (Sims 1989); exchangeable Ca^{2+}, Mg^{2+}, and Al^{3+} were extracted with 1 M KCl (Lin and Coleman 1970; Thomas 1982). Concentration of elements in soil samples was determined by colorimetry (P and Al^{3+}) and atomic absorption (K^{+}, Ca^{2+}, and Mg^{2+}). Soil organic matter (SOM) was measured following the Walkley–Black method according to Nelson and Sommers (1996). Soil bioavailable metals were extracted by diethylene triamine pentaacetic acid (DTPA) (Lindsay and Norvell 1978), and quantified by ICP-OES determination.

Biological and biochemical analyses

Soil microbial biomass C (MBC) was determined by the chloroform-fumigation-extraction method (Vance et al. 1987). Distilled water was added to soil samples (20 g) until they reached 90 % of their water-retention capacity (equivalent to the water content retained in the soil at 6 kPa), and samples were incubated in the dark for 5 days at 28 °C. Subsequently, half of the samples were fumigated (F) at room temperature for 48 h in a desiccator containing 20 ml of ethanol-free chloroform. During this period, unfumigated controls (UF) were kept at 28 °C. Organic C was extracted by adding 50 ml 0.5 M K_2SO_4 solution to 20 g of soil, and the amount of C was determined by humid digestion in acid medium containing 0.4 M $K_2Cr_2O_7$. MBC was calculated by the difference between organic C extracted from F and UF controls using a Kec of 0.35.

β-Glucosidase (E.C. 3.2.1.21), acid phosphomonoesterase (E.C. 3.1.3.2) and arylsulfatase (E.C.3.1.6.1) activities were determined according to Tabatabai (1970), except that toluene was omitted in the assays. Analyses were conducted with three replications and with a non-substrate control.

Soil chemical and microbiological properties were analyzed by one-way analysis of variance (ANOVA). Significant differences between means were assessed

by Duncan's test at the 5 % level of significance. ANOVA and Duncan's tests were performed using R package *laercio* version 1.0-1 (Silva 2010). Principal component analysis was used to assess the effects of the different soil types on the soil microbiological functioning (SMF), described here by the simultaneous analysis of the four biological variables studied (MBC, β-glucosidase, acid phosphomonoesterase and arylsulfatase) (de Carvalho Mendes et al. 2012). Prior to analysis, the data for each variable was normalized by dividing their respective values by the sum of their measurements, in order to avoid the differences in their units. This data transformation to values that are relative to their totals also assigned a similar weight to all variables (de Carvalho Mendes et al. 2012). The normalized data matrix composed of the four measured variables was called the SMF matrix. Ordination was performed using the PC-ORD v. 5.0 (McCune and Mefford 1999) in autopilot mode with the "slow and thorough" option selected. The number of dimensions to be interpreted was chosen according to the stress and stability of the graphical solutions. Variations in SMF between soils were also evaluated by calculating Pearson correlation coefficients between individual values of microbiological and chemical variables and PCA scores (axes 1 and 2).

16S rRNA gene library construction and sequencing of clones

Three 16S rRNA gene libraries were constructed using soil samples from Sites 1, 2, and from the adjoining non-serpentine Cerrado site. DNA was extracted using the MOBIO Ultra Clean Soil DNA Isolation Kit (MoBio Laboratories Inc., Carlsbad, USA). PCRs were performed independently for each plot, using primers 27F and 1492R (Lane 1991), following the protocol described in Mirete et al. (2007): PCR mixtures contained 10 ng of metagenomic DNA, 250 μM dNTPs, 1.5 mM $MgCl_2$, 200 nM of each primer, 2.5 U Taq DNA polymerase, and 1X reaction buffer (Invitrogen), in a final volume of 50 μl. The amplification program included 1 step of 5 min at 95 °C; 35 cycles of 45 s at 95 °C, 45 s at 44 °C, and 2 min at 72 °C; and a final step of 10 min at 72 °C. Two PCRs were performed, and amplification of the 16S rRNA gene was verified by 1 % agarose gel electrophoresis. Products were then pooled, each pool including amplified fragments from three plots, and from two independent reactions. Amplified products were purified using PureLink PCR purification kit (Invitrogen), cloned into plasmid vector pGEM-T Easy (Promega), and transformed into *Escherichia coli* DH10B (Invitrogen) by electroporation. Positive clones were stored in 96 well plates in LB medium containing 0.1 mg/ml of ampicillin and 20 % of glycerol. Libraries were stored at −80 °C.

Plasmids were extracted using alkaline lyses technique (Sambrook and Russel 2001). Approximately 100 clones from each library were sequenced in a 3130xL Genetic Analyzer (Applied Biosystems) at Universidade Católica de Brasília, Brazil, using primer 27F.

Sequence processing and analyses

Sequences were trimmed using *phred* and *lucy* (Chou and Holmes 2001), and vector masked with *cross_match*. Processing of sequences followed standard operational procedures described in Schloss et al. (2011) using mothur (http://www.mothur.org/wiki/454_SOP, accessed in September 1, 2014). In summary, unique sequences were aligned using the SILVA database as a reference; sequences were screened and filtered so that the alignment overlapped in the same region of the 16S rRNA gene; columns containing only gaps were removed; chimeras were detected using the *chimera.uchime* command. Clustering of OTUs using different distance cutoffs (unique OTUs, and OTUs defined by clustering sequences with <3, <10 and <20 % sequence dissimilarities) was performed. Collector's curves at these cutoffs were obtained for estimators of richness (observed richness; Chao1, ACE, and Jacknife estimators), diversity (sampling coverage; Shannon's H', Simpson's D and inverse Simpson indexes), shared community richness (observed shared richness; shared Chao1 and shared ACE estimators) communities' membership (Jaccard similarity coefficient based on observed richness) and structure (abundance-based Jaccard similarity coefficient, abundance-based Sorenson similarity coefficient, Bray–Curtis similarity coefficient, Canberra, Gower, Hellinger, Manhattan, Morisita-Horn, Odum, Soergel, Θ_N, and Θ_{YC} coefficients), all of which are implemented in mothur. Rarefaction curves for the number of observed OTUs using the same thresholds described above were also calculated. CatchAll (Bunge 2011) was used to obtain

the best parametric estimators of richness for our data. OTU-based analyses for alpha and beta diversity parameters were performed at the 3 % distance level. Differences in bacterial community structures were investigated with OTU-based methods, using abundance-based Jaccard and Sorenson dissimilarity indexes (Chao et al. 2004), and the Yue and Clayton dissimilarity coefficient; $\int$-libshuff (Schloss et al. 2004), and tree-based methods Parsimony, Unweighted UniFrac (Lozupone and Knight 2005) and weighted UniFrac (Lozupone et al. 2007) were also applied. Principal coordinate analysis (PCoA) was also applied for visualization of beta-diversity information, and statistical significance of the observed separation of samples was measured with AMOVA in mothur. Correlations between soil chemical data, MBC, and enzyme activities, and the PCoA axes were obtained. Biplots were built using R packages *calibrate* (Graffelman 2013) and *shape* (Soetaert 2014). Correlation between differences in bacterial community structures and differences in SMF was checked with a Mantel test using R package *ade4* (Dray and Dufour 2007).

Sequences were also analyzed with the Classifier tool available at RDP (Cole et al. 2009), using a 90 % threshold, for preliminary phylogenetic affiliation. The Seqmatch tool was used so that a set of best hits, including sequences from both type isolates and environmental samples, could be selected. Aligned sequences were downloaded and Neighbor-Joining trees for each sampling area were built using Jukes-Cantor distances in MEGA 6.06 (Tamura et al. 2013), with 200 bootstraps.

An attempt to compare the bacterial communities from serpentine soils in Brazil to communities from other serpentine sites in the world (Idris et al. 2004; Oline 2006; Herrera et al. 2007), as well as with bacterial communities from non-serpentine Cerrado soils (Araujo et al. 2012) was carried out. However, because these studies used different 16S rRNA gene regions, alignment of sequences as a single dataset was not possible. In order to circumvent this limitation, sequences were classified in mothur using the SILVA database as a reference, in order to create an abundance matrix for each site using the taxonomic classifications of sequences. This matrix was analyzed in R (R Core Team 2014) using principal component analysis.

Results

Chemical and microbiological soil properties

Chemical properties of the ultramafic soil samples are presented on Table 1. Soil pH values were 6.19 in Site 1, and 6.49 in Site 2. Their exchangeable acidities (H + Al) ranged from 3.24 $cmol_c\ dm^{-3}$ in Site 1, to 6.46 $cmol_c\ dm^{-3}$ in Site 2. Site 1 presented higher values of SOM, P, K, and Ca contents, when compared to Site 2, while the latter presented a higher value of Mg. The Cerrado soil site presented lower pH and Mg content, but higher exchangeable acidities (H + Al), and P and K contents, when compared to the two ultramafic samples.

Table 2 shows the metal availability for the three sites. The available Ni content was the most abundant among the investigated metals, with the highest value (603.53 mg kg^{-1}) in Site 1, whereas available Cu, Fe, Mn, and Zn contents were higher in Site 2 than in Site 1 soils. The Cerrado site soil had the lowest concentration of bioavailable Ni (5.20 mg kg^{-1}), whereas those of Cu, Fe, Mn and Zn were higher in the Cerrado than in the ultramafic soil samples.

Table 3 shows results for MBC and enzyme activities. Site 1 soil showed higher values of MBC and enzyme activities than the Site 2 soil. On average, MBC, β-glucosidase, acid phosphomonoesterase and arylsulfatase activities were 2.2, 3.4, 3.3, and 4.6 times higher in Site 1 than in Site 2, respectively.

Although Site 1 and the non-ultramafic Cerrado soil presented similar contents of MBC and β-glucosidase, their SOM contents and activities of arylsulfatase and acid phosphomonoesterase were different (Tables 1, 3). SOM contents, arylsulfatase and acid phosphomonoesterase activities were on average 1.3, 2.7 and 1.4 times higher in Site 1 than in the non-ultramafic Cerrado soil (Tables 1, 3). On the other hand, Site 2, with lower levels of available Ni, presented a reduced MBC value and enzyme activities in relation to the non-ultramafic Cerrado soil (Table 3). The only exception was arylsulfatase activity, for which the difference between Site 2 and Cerrado was not statistically significant (Table 3). These differences were more accentuated for MBC, which was 4.0 times greater in the Cerrado soil (Table 3). SOM contents in the Site 2, Cerrado and Site 1 soils were on average 18, 40 and 64 g kg^{-1}, respectively (Table 1).

Table 1 Chemical properties of two ultramafic soils and a non-ultramafic Cerrado soil

Sites	pH (H_2O)	H + Al ($cmol_c\ dm^{-3}$)	Mg ($cmol_c\ dm^{-3}$)	Ca ($cmol_c\ dm^{-3}$)	P ($mg\ dm^{-3}$)	K ($mg\ dm^{-3}$)	SOM (%)
Site 1	6.19 b	6.46 b	5.19 b	1.53 a	0.6 b	59 a	64 a
Site 2	6.49 a	3.24 c	6.95 a	0.82 b	0.3 c	31 b	18 c
Cerrado	5.86 c	9.79 a	0.15 c	nd	1.9 a	67 a	40 b

Values were the means of three replicates

Means in the same column followed by the same letter were not significantly different by Duncan's test ($p < 0.05$). Samples were collected from 0 to 10 cm depth; *nd* not detected

Table 2 Extractable metals in two ultramafic soils and a non-ultramafic Cerrado soil

Sites	Cu ($mg\ kg^{-1}$)	Fe ($mg\ kg^{-1}$)	Mn ($mg\ kg^{-1}$)	Ni ($mg\ kg^{-1}$)	Zn ($mg\ kg^{-1}$)
Site 1	1.36 c	6.72 c	1.46 c	603.53 a	0.15 c
Site 2	1.89 b	23.85 b	19.70 b	134.66 b	0.41 b
Cerrado	2.97 a	66.94 a	88.91 a	5.20 c	0.61 a

Values were the means of three replicates

Means in the same column followed by the same letter were not significantly different by Duncan's test ($p < 0.05$). Samples were collected from 0 to 10 cm depth

Table 3 Microbial biomass C (MBC), β-glucosidase, arylsulfatase and acid phosphomonoesterase activities, in two ultramafic soils and a non-ultramafic Cerrado soil

Sites	MBC ($mg\ C\ kg^{-1}$)	β-glucosidase (μg *p*-nitrofenol $g^{-1}\ h^{-1}$)	Arylsulfatase (μg *p*-nitrofenol $g^{-1}\ h^{-1}$)	Acid phosphomonoesterase (μg *p*-nitrofenol $g^{-1}\ h^{-1}$)
Site 1	679 a	80 a	213 a	1108 a
Site 2	200 b	37 b	46 b	334 c
Cerrado	795 a	62 a	76 b	674 b

Values were the means of three samples

Means in the same column followed by the same letter were not significantly different by Duncan's test ($p < 0.05$). Samples were collected from 0 to 10 cm depth

Microbiological functioning

The graphic representation of the soil microbiological functioning (SMF), expressed as the multivariate pattern of soil microbial biomass C and the activity of enzymes related to the C, P and S cycles, is shown in Fig. 1. A clear and statistically significant separation between Sites 1, 2 and Cerrado soils was observed ($p = 0.001$). A gradient between different soils can be observed along Axis l (from Site 2 through Cerrado to Site 1). 99.1 % of the total variability found in the SMF was represented in the PCA, 95.4 % of which was associated with Axis 1.

Table 4 shows Pearson's correlation coefficients between microbiological and chemical data (presented in Tables 1, 2, 3) and the two axes of the PCA in Fig. 1. Microbial properties were predominantly correlated with Axis 1 of the PCA, although MBC was also correlated with Axis 2 (Table 4). Axis 1 was positively associated with K, SOM and Ni, whose values increased from Site 2 to Site 1 soils (Tables 2, 4). Ultramafic soils (Sites 1 and 2) were separated from the non-ultramafic Cerrado soil along Axis 2. Soil pH and Mg content (whose values decreased from the ultramafic Site 2 and Site 1 soils to the non-ultramafic Cerrado soil) were negatively correlated with this axis,

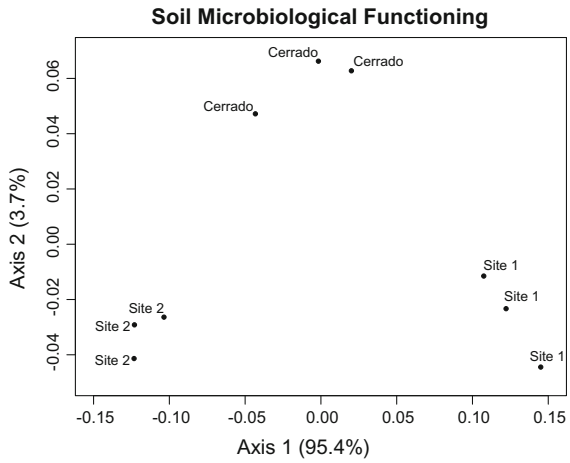

Fig. 1 Principal Component Analysis plot of two ultramafic soil sites (three replicas each) with respect to SMF properties (MBC, β-glucosidase, arylsulfatase, and acid phosphomonoesterase activities). The proportions of variance represented by each axis are shown in *parenthesis*

whereas contents of P, K, H + Al, and available Cu, Fe, and Mn (which increased from the ultramafic Site 2 and Site 1 soils to the non-ultramafic Cerrado soil) were positively correlated with this axis. The Site 2–Cerrado–Site 1 gradient described along Axis l, and the significant correlations found between the microbial properties and this axis, showed that these properties increased in this direction, following the same trend observed for the SOM content in these soils.

Bacterial community diversity and structure

Sequencing of clones from the three 16S rRNA gene libraries generated a total of 281 sequences with an average size of 594 bp (96 from Site 1, 91 from Site 2, and 94 from Cerrado soil), which were deposited in Genbank under accession numbers KM280102–KM280382. After alignment, removal of poor quality sequences, clustering, and chimera detection, the total number of sequences was reduced to 273, 229 of which were unique. Neighbor-joining trees including sequenced clones and best hits from the RDP database were built for each library. Figure 2 shows the tree built for Site 1, and the presence of nine phyla, two of which—Acidobacteria and Actinobacteria—were the most abundant in Sites 1 and 2 (online resource 2). In the Cerrado soil, Acidobacteria was also the most abundant phylum, followed by Proteobacteria (online resource 3).

Table 4 Pearson's correlation coefficient between microbiological and chemical properties and axis 1 (PC 1) and 2 (PC 2) of the principal component analysis plot presented in Fig. 1

Variable	Axis	
	PC1	PC2
pH H_2O	−0.39 ns	−0.94***
Ca	0.50 ns	−0.65 ns
Mg	0.33 ns	−0.84**
P	0.03 ns	0.97***
K	0.68*	0.78*
H + Al	0.42 ns	0.94***
SOM	0.98***	0.24 ns
Cu	−0.39 ns	0.79**
Fe	−0.32 ns	0.83**
Mn	−0.29 ns	0.86**
Ni	0.79**	−0.44 ns
Zn	−0.59 ns	0.61 ns
MBC	0.69*	0.82**
β-glucosidase	0.92***	0.34 ns
Arylsulfatase	0.96***	−0.09 ns
Acid phosphomonoesterase	1.00***	0.18 ns

Samples were collected at the 0–10 cm depth

ns non-significant; *, ** and *** significant at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level, respectively

Collector's curves generated for the available richness estimators in mothur showed that none of them were suitable for comparing richness estimates between sites at a 3 % distance level. Indeed, no significant differences in Chao1 and ACE richness estimators were found between Sites 1, 2 and Cerrado (data not shown). We used CatchAll (Bunge 2011) as an alternative to look for the best richness estimator for our datasets. The best results followed a single exponential-mixed Poisson parametric model, but differences between sites at a 3 % distance level were still not statistically significant (Fig. 3a). Shannon diversity index (H′) showed there was little difference in OTU diversity between sites, with a significant difference only between Site 1 and Cerrado (Fig. 3b). No significant differences in community diversity were found with the Simpson index (D) (Fig. 3c). The values of the reciprocal of Simpson's index were 141 for Site 1, 126.32 for Site 2, and 48.25 for Cerrado. Rarefaction curves of the number of observed OTUs

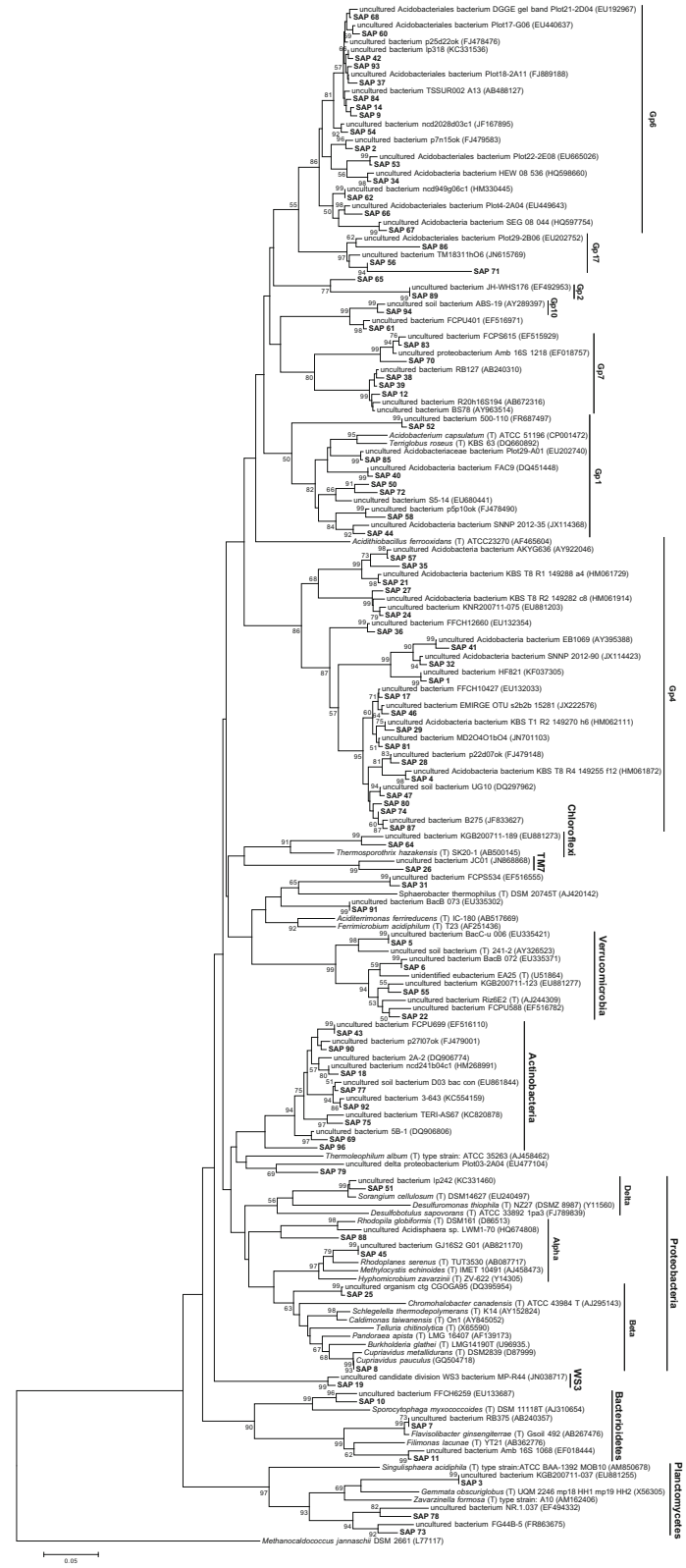

Fig. 2 Neighbor-joining tree based on 16S rRNA gene sequences from Site 1 clones (in *boldface*) and their closest hits from RDP (with their corresponding accession numbers in *parenthesis*). Bootstrap values above 50 are shown. The tree was rooted with *Methanocaldococcus jannaschii* as an outgroup

as a function of sampling effort showed a larger number of OTUs at a 3 % distance cutoff in Sites 1 and 2 than in Cerrado (online resource 4).

Shared richness results showed that Sites 1 and 2 shared 7 OTUs, Cerrado and Site 1 shared 6 OTUs, and Cerrado and Site 2 shared 3 OTUs (Fig. 4a). Shared Chao1 richness estimates followed this trend, and were higher between Sites 1 and 2, followed by Cerrado and Site 1, and Cerrado and Site 2 (Fig. 4a). Abundance-based dissimilarity coefficients (Chao et al. 2004), show that Cerrado and Site 1 were less dissimilar regarding their relative abundances, followed by comparing Sites 1 and 2, and finally Cerrado and Site 2 (Fig. 4b). The Θ_{YC} coefficient showed no statistically significant differences between pairwise comparisons in community structure (Fig. 4c).

Differences in the structure of bacterial communities were also investigated with $\int$-libshuff (Schloss et al. 2004), which showed there were significant differences between Cerrado and Site 1 ($p = 0.0015$), and Cerrado and Site 2 ($p = 0.0001$), but that Sites 1 and 2 were not different (Table 5). Phylogenetic methods relied on the construction of a neighbor-joining tree with the *neighbor* program in the Phylip package, using pairwise distances between aligned DNA sequences generated with the *dist.seqs* command in mothur. The Parsimony method showed similar results to those given by $\int$-libshuff, i.e. significant differences between Cerrado and Site 1, and Cerrado and Site 2, but no difference between Sites 1 and 2. This was also the result given by Unweighted Unifrac (Table 5). When evaluated with weighted Unifrac, on the other hand, all pairwise comparisons yielded significant results (Table 5), meaning that community structures in Sites 1 and 2 were also statistically different.

We used pairwise Θ_{YC} distance data between sites to build a PCoA plot and visualize β-diversity information (Fig. 5, see below). In order to test the statistical significance of the spatial separation between sites in the PCoA plot, Sites 1 and 2 were labeled as "Serpentine" and Cerrado as "Non-Serpentine", and differences between these groups of samples were tested with AMOVA in mothur. As expected from the previous result with Θ_{YC}, the separation between these groups of samples was not statistically significant ($p = 0.665$). One hundred percent (100 %) of the total variability found in Θ_{YC} distance was represented in the PCoA, 54.83 % of which was associated with Axis 1, and 45.17 % with Axis 2 (Fig. 5). The correlation between chemical and microbiological functioning data shown in Tables 1, 2 and 3, and the two PCoA axes was also tested. Biplots showing these significant correlations and the PCoA ordination of the three sites can be seen on Fig. 5. Pearson correlation coefficients showed that soil pH and Mg values were negatively correlated with Axis 1, while H + Al, P, and K were positively correlated with this axis; Zn values were negatively correlated with Axis 2, while Ni values were positively correlated with this axis; correlations with SMF data showed that MBC was positively correlated with Axis 1, while Arylsulfatase, and Acid Phosphomonoesterase, were positively correlated with Axis 2. Interestingly, SOM

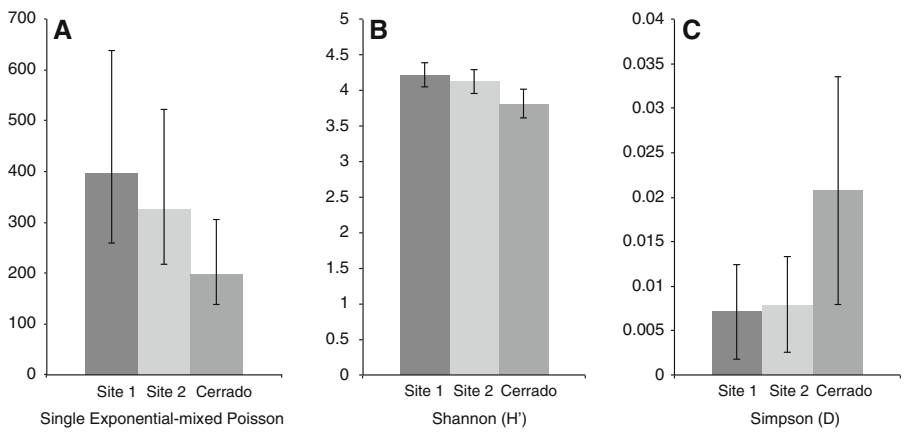

Fig. 3 **a** OTU-based richness estimate (single exponential-mixed Poisson) for Sites 1, 2 and Cerrado bacterial communities; **b**, **c** OTU-based diversity (Shannon's H′ and Simpson's D, respectively) estimates for Sites 1, 2 and Cerrado bacterial communities

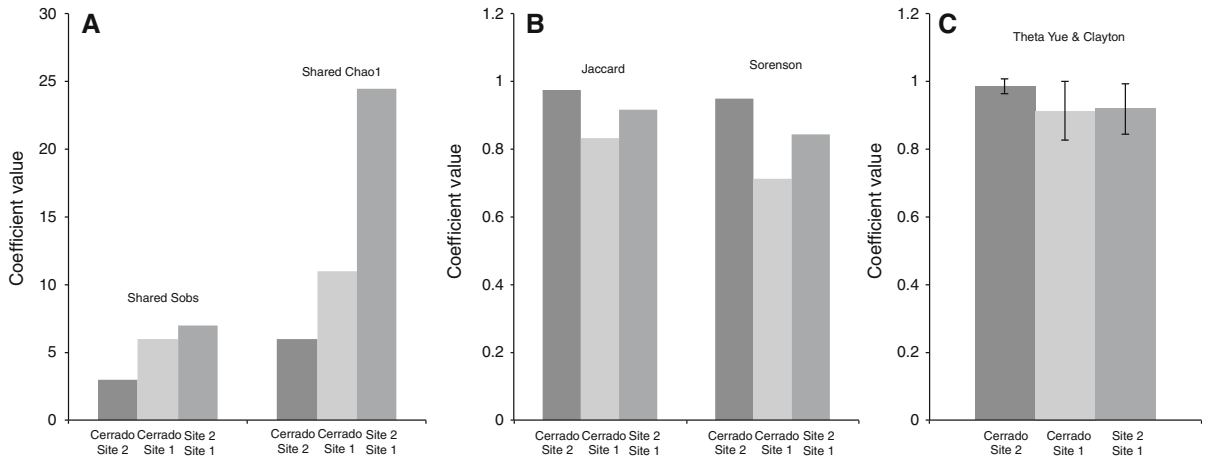

Fig. 4 **a** Pairwise comparisons of shared richness (shared number of observed OTUs, and shared Chao1 richness estimator); **b**, **c** pairwise comparisons of shared structure (abundance based Jaccard and Sorenson similarity coefficients; Θ_{YC} dissimilarity coefficient, respectively) of bacterial communities

Table 5 Tests of differences between bacterial community structures in sites 1, 2 and Cerrado

Method	Comparisons	Score	*p* value
Parsimony	**Cerrado–Site 2**	25	0.001
	Cerrado–Site 1	30	0.001
	Site 2–Site 1	52	0.333
Unweighted Unifrac	**Cerrado–Site 2**	0.8845	<0.001
	Cerrado–Site 1	0.8470	0.004
	Site 2–Site 1	0.8207	0.201
Weighted Unifrac	**Cerrado–Site 2**	0.6047	<0.001
	Cerrado–Site 1	0.5775	<0.001
	Site 2–Site 1	0.3652	<0.001
∫-libshuff	**Cerrado–Site 2**	0.005	0.001
	Site 1–Cerrado	0.005	0.0015
	Site 1–Site 2	0.0007	0.2628

Statistically significant differences are depicted in bold

contents were not correlated with any axis. The Mantel test showed no correlation between Θ_{YC} distances and differences in SMF.

PCA of community diversity of all serpentine and Cerrado rRNA gene libraries available in Genbank showed three distinct groups of samples (Fig. 6). The first cluster contained all Cerrado samples, including the non-serpentine Cerrado soil from Barro Alto and other native Cerrado sequences obtained in previous studies (Araujo et al. 2012). This cluster is correlated with the presence of sequences belonging to the acidobacteria subgroup GP1. A second cluster contained both serpentine soil samples from Barro Alto, which is correlated with acidobacteria subgroups 4 and 6. The third cluster includes samples from serpentine soils from different locations, although the sample originated from mine spoils in New Caledonia (MIN) was more distant to others gathered in this cluster (Fig. 6).

Discussion

Given the unusual chemical characteristics of serpentine soils, one would expect that microbial communities subjected to such harsh conditions would show signs of stress. Indeed, negative effects of an increase of metal biological availability on metabolic activities and diversity of these microbes have been observed (Hattori 1992; Sandaa et al. 1999; Kozdrój and van Elsas 2001; Stefanowicz et al. 2008). However, we have not found that microbial populations of undisturbed serpentine soils were affected by their high Ni concentrations. This raises the question of whether the interpretation of soil quality indices is suitable for undisturbed environments where metal availability is not caused by pollution. Differences in SMF were more related to organic matter content than to available Ni content of these soils. For instance, the gradient Site 2–Cerrado–Site 1 observed in axis 1 of Fig. 1 (which explained 95.4 % of the total variability on SMF), was clearly associated with increasing SOM

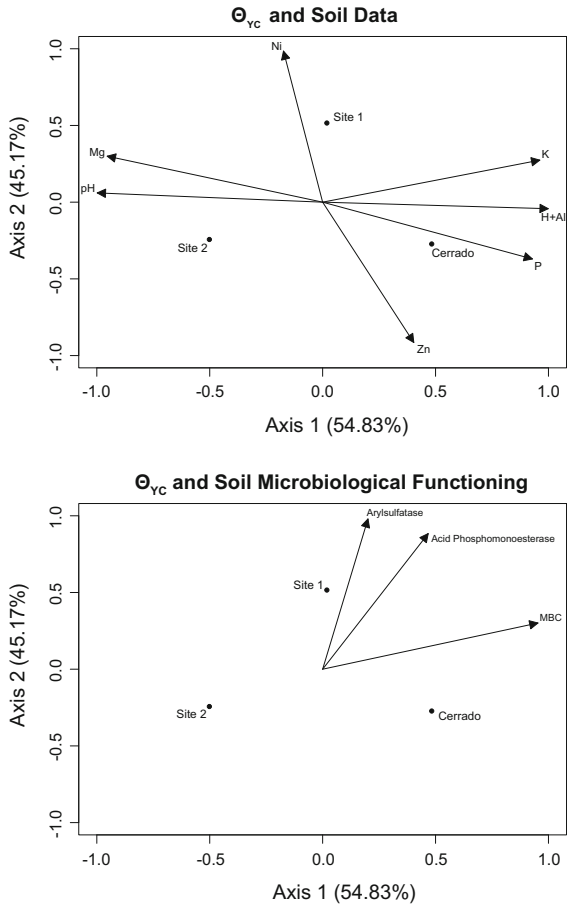

Fig. 5 PCoA ordination of Θ_{YC} distances between Sites 1, 2 and Cerrado based on 16S rRNA gene sequence data. *Arrows* depict significant Pearson correlation coefficients between soil chemistry data (*top chart*) and SMF data (*bottom chart*) with the two PCoA axes

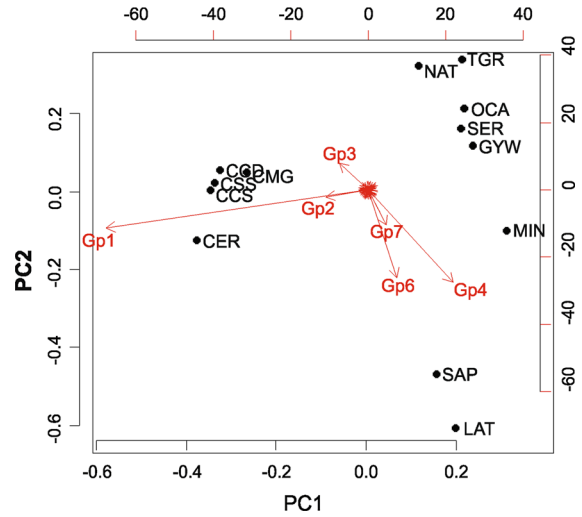

Fig. 6 Principal component analysis based on taxonomic classification of 16S rRNA gene sequences from bacterial communities from Site 1 (*SAP*), Site 2 (*LAT*) and non-serpentine Cerrado (*CER*). *Arrows* depict correlations between abundance of identified sequences with the PCA axes. The following datasets from serpentine sites worldwide and Cerrado soils in Brazil are also included: (*OCA*) Oregon and California, USA (Oline 2006); (*TGR*) *Thlaspi goesingense* rhizosphere, Austria (Idris et al. 2004); (*NAT*) Native vegetation, (*SER*) *Serianthes calycina* rhizosfere, and (*GYW*) *Gymnostoma webbianum* rhizosfere, all from New Caledonia (Herrera et al. 2007); (*CCS*) Cerrado Campo Sujo, (CCD) Cerrado Denso (*CCD*), (*CMG*) Mata de Galeria (*CMG*), (*CCS*) Cerrado *Sensu Stricto* (Araujo et al. 2012)

contents. Although MBC content was similar between the ultramafic soil with high Ni and the non-ultramafic Cerrado soil, the former presented higher enzyme activities and SOM content. Schipper and Lee (2004) observed similar results on serpentine soils of West Dome, New Zealand: MBC, respiration, catabolic evenness and respiratory quotient were not correlated to either total or extractable Cr, Ni or Mg, whereas microbial biomass and basal respiration were significantly correlated with total C content. Litter decomposition in serpentine soils can be influenced either indirectly or directly by the effects of this type of environment on the soil decomposer communities (Kazakou et al. 2008). However, the hypothesis regarding the indirect influence of serpentine soils on litter decomposition has not been investigated in natural serpentine ecosystems (Kazakou et al. 2008).

Considering that soils under native climax vegetation are rapidly disappearing in the Cerrado (51.5 % of the original vegetation was present in 2009) and that it is not possible to estimate the value of bioindicators of soil quality in stored soils (Gil-Sotres et al. 2005), this study adds benchmark information on soil microbiological functioning for a unique type of environment within the Cerrado biome. Recently, de Carvalho Mendes et al. (2012) reported that different Brazilian Cerrado physiognomies under natural vegetation are major determinants of soil biological functioning, calculated by analyzing microbial biomass C, soil basal respiration and the activity of enzymes linked to the C, P and S cycles (β-glucosidase, acid phosphomonoesterase and arylsulfatase, respectively). Their results also showed that differences detected in SMF were more evident in the topsoil than in the deeper layers.

Analysis of 16S rRNA gene libraries from the three sites showed the predominance of Acidobacteria. Similar studies in serpentine soils from geographically distant areas showed a predominance of Actinobacteria and Proteobacteria (Idris et al. 2004; Mengoni et al. 2004; Oline 2006), with the exception of a study from mine spoils and revegetated soils in New Caledonia (Herrera et al. 2007). Their study showed a predominance of Acidobacteria in mining sites (without any vegetation), but not in sites revegetated with native plants, where Proteobacteria was the most abundant phylum. Prevalence of Acidobacteria in bulk soil has been reported in various non-serpentine Cerrado samples (Araujo et al. 2012). However, subgroups of Acidobacteria present in these serpentine soils (subgroups 4 and 6) differed from those of non-serpentine Cerrado soils from other areas. This result is consistent with a positive correlation of relative abundance of Acidobacteria subgroups 4 and 6 with higher soil pHs, found in a previous study of soil acidobacterial diversity (Jones et al. 2009).

Statistically significant differences in OTU-based richness estimates could not be detected, at least with the available number of sequenced clones. This was also true for diversity estimates such Simpson's D and Shannon's H′ (for which a significant difference was detected only between Site 1 and Cerrado). A higher number of sequenced samples would probably reduce the error associated with these estimates, and allow the detection of significant differences. Herrera et al. (2007) used the reciprocal of Simpson's D as a measure of dominance levels in bacterial communities, and as suggested by Zhou et al. (2002), the Cerrado soil sample was the only one presenting signs of a dominance profile (with a value of 1/D below 50). Rarefaction curves showed that although richness at the phylum level (20 % dissimilarity cutoff) was almost saturated, this was not true for species richness (3 % dissimilarity cutoff) with this sampling depth.

Shared richness data showed that Sites 1 and 2 shared more OTUs than any other pairwise combination of samples. Although no significant differences were found in pairwise comparisons of Θ_{YC} distances, phylogenetic based comparisons such as $\int$-libshuff, Parsimony, and unweighted Unifrac showed statistically significant differences between Site 1 and Cerrado, and Site 2 and Cerrado. This indicates that bacterial communities from serpentine sites are more similar to each other than to that from a non-serpentine adjoining soil, a result that is similar to what has been reported by Oline (2006). Interestingly, weighted Unifrac, a quantitative measure of beta diversity, also detected a significant difference between the two serpentine sites, indicating that the relative abundance of lineages is different between the two serpentine sites.

While differences in SMF have been more correlated to SOM than to Ni contents, this was not true for differences in bacterial community structures as measured by Θ_{YC}, for which no correlations with SOM were detected. Also, no correlation was found between differences in SMF and differences in bacterial community structures, although activities of two enzymes and MBC were significantly correlated with the PCoA ordination of Θ_{YC} data. Changes in biomass and activities of soil microbial communities are not necessarily accompanied by changes in their phylogenetic structure (Stark et al. 2008; Peixoto et al. 2010). Regarding soil chemistry, a study by Daghino et al. (2012) found that mineral and chemical differences in serpentine substrates were not correlated with fungal diversity, measured by 454 pyrosequencing and DGGE profiling.

Schipper and Lee (2004) suggested that metals can regulate the heterotrophic microbial community structures of serpentine soils through their effects on plant communities, which determine C amounts by rhizodeposition and litter inputs. Microbial community structures of soils covered by different plant communities or subjected to distinct management practices are indeed different (Ohtonen et al. 1999; Pennanen 2001; Quirino et al. 2009). A joint analysis including data on plant communities from these same areas would provide a full picture of the relationships taking place in these areas.

Conclusion

Our results show that the multivariate pattern of soil microbial biomass C and the activity of enzymes related to the C, P and S cycles were not affected by high Ni concentrations. The changes in SMF observed in this study were more related to organic matter content than to available Ni content. Phylogeny-based

results indicated differences in bacterial community structures between the non-serpentine Cerrado and the two serpentine sites, and also that abundance of bacterial lineages between Sites 1 and 2 was different. No correlation between differences in SMF and bacterial community structures were detected. The establishment of different plant communities may influence the structure and functioning of the bacterial communities characterized in this study. Further analyses including data from the composition, diversity and structure of these plant communities would provide valuable information on their relationships with soil composition and microbial community diversity and function in ultramafic environments.

Acknowledgments We thank Clodoaldo A. de Sousa, Lucas F.L.S. Rolim, Franciele Schlemmer, Leandro M. de Souza, and Milene R. Ribeiro, for their assistance during this study. We thank Fabiana de Gois Aquino for kindly providing images of the samples sites. We also thank Anglo American and their team at the Barro Alto plant for their support. This work was partially financed by, Embrapa Macroprograma 2—Grant# 02.07.01.007.00.00, Embrapa Macroprograma 3—Grant# 03.09.06.016.00.00, and the CNPq (National Council for Scientific and Technological Development) REPENSA call (562433/2010-4).

Conflict of interest The authors declare that they have no conflict of interest.

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