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REGULAR ARTICLE

The influence of litter quality on the relationship between vegetation and below-ground compartments: a Procrustean approach

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Abstract
Aims We used a Procrustean superimposition approach associated with regression analysis to test hypotheses regarding the relationship between plant communities and distinct below-ground compartments—soil chemistry (SC) and soil microbial activity (SMA). Additionally, we evaluated litter chemical quality as an interface between the above and below-ground compartments.
Methods Plant community, and soil chemical and biochemical data from three post-mining degraded sites under reclamation and from one nearby forest site in the Brazilian Amazon Basin were analyzed.
Results All studied sites presented distinct plant community, litter quality, SC and SMA. Plant community consistently affected the below-ground variation in both SC and SMA compartments. The influence of litter quality was greater in the plant community versus SMA relationship than in the plant community versus SC. Nevertheless, the SC affected significantly the SMA, but without influence of litter quality.
Conclusions Differently from previous studies, our findings suggest that plant community and soil chemistry can affect the soil microbial activity independently. Specifically for our study area, these results point to a rupture of the 'in nested' structure of the causal relationship between changes in vegetation, changes in the chemical litter quality, changes in the SC and the response of SMA.

Keywords Plant community · Procrustean statistics · Soil microbial activity · Soil chemistry · Association metric

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Introduction

Alterations in the litter quality as a function of changes in plant communities are argued to be the main link between

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the above-ground (AG) and below-ground (BG) compartments (Bardgett and Wardle 2010; Merilä et al. 2010; Orwin et al. 2010; Lamb et al. 2011; Meisner et al. 2012). The abundance of plants, or its derivatives (e.g., coverage and relative abundance), is among the most common features of the plant community used to assess the effect of plant diversity on aspects of the microbial community, such as its composition and functioning (Singh et al. 2008; Lamb et al. 2011; Mitchell et al. 2010b; 2012).

There are a substantial number of studies using abundance data to understand the functional significance of the relationship between the species composition and functional aspects of the BG compartment (Hooper and Vitousek 1997; Merilä et al. 2010; Mitchell et al. 2010a, 2012). The justification for using abundance data is that by taking into consideration the number of individuals per species they represent the weighting of the richness in terms of biomass, which is believed to represent biodiversity and its impacts on ecosystem services more reliably (Grime 1998).

Along with vegetation, the variation in soil chemistry is also reported to be one of the important drivers of the microbial community in the soil (Wardle 1992; Mitchell et al. 2010a, 2012). For example, it has been shown that changes in the functional aspects of below-ground communities are accompanied by variations in pH (Högberg et al. 2007; Rousk et al. 2009; Jesus et al. 2009), the level of nitrogen and total carbon (Williamson et al. 2005; Högberg et al. 2007) and the concentration of heavy metals (Zhang et al. 2010). Nevertheless, the literature shows that in many cases the capacity of soil chemistry to cause changes in aspects of the microbial community is derived from the influence of vegetation via alteration of the litter's chemical quality (Mitchell et al. 2010a, 2012; Orwin et al. 2010; Meisner et al. 2012), leading to the hypothesis that the plant community is able to summarize the main drivers of changes in the microbial composition and functioning in the soil, including the soil chemistry itself (Mitchell et al. 2010a).

In ecology, including soil ecology, the use of techniques for multivariate ordination of different ecosystem compartments is widely disseminated (Legendre and Legendre 1998). Ordination is defined as the description of the variation of observations over an environmental gradient within a reduced space, so that the distances in this space reflect the ecological distances based on a set of specific descriptors. Therefore, the use of statistical approaches that permit assessing the degree of direct relationship between the variations of different ecosystem compartments can be important to help answer some questions. One of them is to what extent the variation of the plant community and the variation of soil chemistry and soil microbial activity are directly related.

A potential statistical approach to answer that question, still not widely disseminated in the field of soil ecology, is the Procrustes analysis or Procrustean superimposition (Peres-Neto and Jackson 2001). This analysis is based on searching for the optimal fit between the ordination solutions and raw data matrices, or dissimilarity matrices (Peres-Neto and Jackson 2001). This optimal fit is attained by summing the squares of the differences between corresponding coordinates (objects of data matrix) in two ordination solutions or two data matrices (Cleary et al. 2012). The significance of the results can be assessed by the permutation test (Peres-Neto and Jackson 2001). One of the advantages of Procrustes analysis is the possibility of extracting the association metric in the form of a vector and submitting it to various statistical procedures (Peres-Neto and Jackson 2001), including regression analysis (Singh et al. 2008) and means testing (Peres-Neto and Jackson 2001).

In this work we report the use of Procrustean superimposition to evaluate the extent to which the variation of plant communities from a rain forest environment is directly related to the variation of the below-ground compartments, that is, chemistry and microbiological soil properties. Through extensive simulations of the Procrustean relationship and subsequent regression analyses and means testing, we tested three hypotheses: 1) the variation in the composition of plants (abundance) is related consistently with the variation of both chemical and microbial activity soil compartments; 2) the relationship between above ground (plant community) and below ground (soil chemistry and microbial activity) compartments is driven by the chemical quality of the litter; and 3) the variation of the soil microbial activity is consistently related to the variation of soil chemistry, with this relationship being influenced by the chemical quality of the litter, that is, by the vegetation.

Material and methods

This study was conducted at sites within a single open pit iron ore mining area, located within the Carajás

National Forest in the southeast of the state of Pará (05°52′ to 06°33′S and 49°53′ to 50°45′ W), in Brazil's Northern region. The climate is humid tropical, with average rainfall of 2,800 mm in the rainy season and 600 mm in the dry season. The average annual temperature is 24.8 °C.

The study was conducted at four sites, three of them former iron ore mining areas with the same type of substrate (overburden), restored at different times and with different management strategies (R2, R3 and R6), and one a primary forest site (For). The three restored sites were all located less than 2 km from each other within the mining area. The total area of the R2, R3 and R6 sites were 0.72, 0.54 and 0.80 ha, respectively.

Brief history of management of the study sites

The recovery of the three mining sites had been carried out in a single period of at most two months, at three different times and using different methods.

The restoration of site R2 (UTM: E 591590 m; N 9331208 m) started in December 2008 and ended in January 2009, exclusively involving spreading a layer approximately 20 cm thick of soil taken from a nearby primary forest area.

The recovery of site R3 (UTM: E 594969 m; N 9327929 m) occurred in November and December 2007, consisting of initial application of 1,500 kg ha^{-1} of dolomitic limestone followed by 600 kg ha^{-1} of NPK (4:14:8 formulation) and seeding with a mix of forage grass seeds (28 kg ha^{-1}) and a mix of native tree and bush species seeds (30 kg ha^{-1}).

Finally, at site R6 (UTM: E 596483 m; N 9329588 m), the restoration took place in November and December 2004. The soil at this site received 1,500 kg ha^{-1} of dolomitic limestone, 400 kg ha^{-1} of natural phosphate, 300 kg ha^{-1} of potassium chloride and 1,500 kg ha^{-1} of chicken manure. The seeding was with a mix of exotic leguminous tree and bush species (280 kg ha^{-1}) and a mix of native tree and bush species (10 kg ha^{-1}).

Soil sampling

The soil samples were collected in August 2010. Three plots (10×20 m, and 20 m apart) were marked out within each site along the largest dimension. Three composite samples were collected from each plot after dividing it in three equal parts. Each composite sample was formed of six single samples collected from random positions and at a depth of 0–10 cm (after removal of the layer of litter) using as soil core probe (5 cm diameter). The samples were then sieved and stored at 4 °C until the moment of the chemical and microbiological analyses. All the analyses were performed within 14 days after the samples had been collected.

The statistical analyses were run with the average of the analytical results obtained for the 3 compound samples.

Study of the plant community

The plant community study was carried out at the same time as the soil sampling. The evaluation consisted of counting and identifying each of the individual plants within each of the three plots (10×20 m) within each site. The herbaceous component (including grasses) had low representation at the sites investigated (<5 % of coverage of each plot), so the vegetation study was based on counting and identifying all the individuals taller than 1.5 m, as suggested by Muller-Dombois and Ellemberg (1974). The plants were identified at the species, genus and family level. At the time of obtaining the soil samples, the plant communities at the different sites presented clear variation in terms of species composition (Fig. 1).

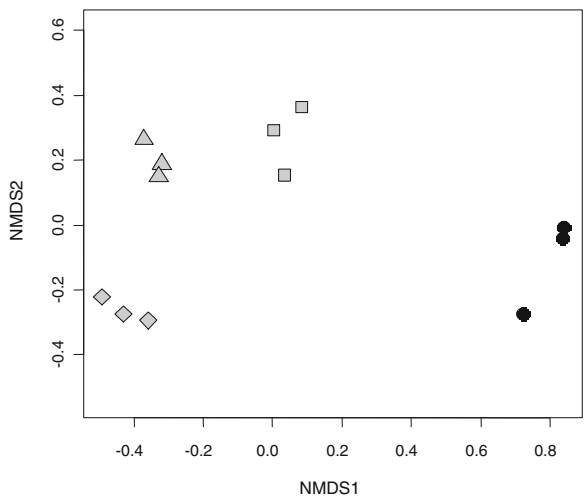

Fig. 1 Variation of the sites regarding composition of the plant community (abundance) at the moment of soil sampling. Non-metric multidimensional scale (NMDS) based in Bray-Curtis distance. Square (site R2), triangle (site R3), diamond (site R6), and circle (Forest)

Site R2 stood out for the high relative dominance of arboreal species belonging to the Urticaceae and Solanaceae families, with the main ones being *Cecropia ficifolia* (>65 %) and *Solanun rugosun* (>75 %). This site also had the least coverage by leguminous species (5.8 %). In contrast, site R3 had the second-highest coverage by leguminous species, at 30 %, but this was mainly due to bushy leguminous species. Site R6 had the most leguminous tree and bush species combined, at 88.7 % of coverage, with the standouts being the species *Senna sylvestris* (63 % of relative dominance) and *Mimosa setosa* (90 % of relative dominance).

Above- and below-ground compartments

The above-ground (AB) compartment was represented by the plant composition, that is, the matrix of abundance data formed by 120 species (Online Resource 1). The below-ground (BG) compartment was divided between the soil chemistry (SC) and the soil microbiology. In this study, we considered the SC as being the set formed by: pH in H_2O; sum of (H^++Al^{+3}); total soil carbon, total soil nitrogen (CHNS/O Elemental Analyzer, PerkinElmer Inc. USA), C:N ratio; extractable phosphorous in Mehlich 1; concentrations of Ca and Mg (in KCl 1M); and potential (pH 7.0) and effective cation exchange capacity. All the soil chemical variables were measured using the standard methods described in Embrapa (1997).

The microbiological properties employed to compose the second below-ground compartment were chosen based on having been previously shown to have a direct and/or indirect relationship with the functioning of the microbial community in the soil and specific microbial groups, potentially affected by aspects of the plant cover (Chaer et al. 2009; Garcia-Palacios et al. 2010; Harris 2003; Ingram et al. 2005; Mukhopadhyay and Joy 2010; Purin and Rillig 2007). For convenience and simplicity, the set of variables forming the microbiological compartment are henceforth called the soil microbial activity (SMA). The microbiological properties measured were biomass carbon, basal respiration, metabolic quotient, fluorescein diacetate hydrolysis, phosphatase activity, β-glucosidase activity, phenol oxidase activity, and levels of soil protein related to glomalin (easily extractable fraction, and total fraction).

The microbial biomass C (MBC) was determined by using the chloroform-fumigation-extraction protocol (Vance et al. 1987). The extract obtained in 0.5M K_2SO_4 solution was analyzed colorimetrically by using potassium permanganate as an oxidant agent (Bartlett and Ross 1988). MBC values were obtained using a K_c value of 0.35 (Anderson et al. 2008). The results were expressed in mg MBC kg^{-1} dry soil. The basal soil respiratory rate (Resp) was determined from substrate/soil incubated with NaOH traps to capture CO_2, followed by titration with HCl (Silva et al. 2007). The respiratory rate was measured for 10 days after incubation in hermetically sealed 2 L flasks kept at 25 °C in the dark. Carbon production from respiration was expressed in μg of CO_2-C kg^{-1} dry soil h^{-1}. The metabolic quotient (qCO_2) was calculated from the Resp/MBC ratio.

Hydrolysis of fluorescein diacetate was determined by incubating the samples for 2 h (37 °C) and determining the amount of fluorescein released, according to Scnhürer and Rosswall (1982). This assay evaluates the overall activity of proteases, lipases, and esterases (Green et al. 2006). All samples were analyzed in duplicate, and the results were expressed in μmol fluorescein g^{-1} soil h^{-1}.

Acid phosphatase and β-glucosidase activities were determined spectrophotometrically according to Tabatabai (1994) and Eivazi and Tabatabai (1988), respectively. The phosphatase method was modified by reducing in ½ substrate/soil and solutions, and by using water instead of a buffered reaction medium. Analyses were run in test tubes and the incubation was in water bath for 1 h at 37 °C. The amount of *p*-nitrophenol formed in the reactions was determined by comparing it to a standard curve with known *p*-nitrophenol levels, and the results were expressed in μmol *p*-nitrophenol g^{-1} dry soil h^{-1}. All samples were analyzed in duplicate.

Laccase activity was determined spectrophotometrically in duplicate, using test tubes with 0.5 g of soil (Sinsabaugh et al. 1999). To each sample, 1 mL H_2O and 1 mL of substrate L-3,4-dihydroxyphenylalanine (L-DOPA) were added, prepared in 5 mM acetate buffer pH 5. For control samples, 1 mL H_2O and 1 mL of acetate buffer were added to the soil. After incubation for 1 h at 30 °C, the reaction was stopped with 1 mL 0.6 % sodium azide (*w*/*v*). Finally, the samples were centrifuged and analyzed spectrophotometrically at 460 nm. The dihydroindole-quinone-carboxylate (DIC) formed was determined according to a micromolar extinction coefficient of 1.6 (Sinsabaugh et al. 1999). Laccase activity was expressed in nmol DIC g^{-1} dry soil h^{-1}.

Total glomalin and easily extractable glomalin protein were extracted and quantified according to Wright and Upadhyaya (1998), and the results were expressed in mg of protein g^{-1} dry soil.

Litter sampling and chemical characterization

Three quadrants of 0.25 m^2 (0.5×0.5 m) were randomly positioned inside each plot at each site for collection and subsequent characterization of the chemical quality of the standing litter (Arato et al. 2003). The litter within a quadrant was collected, dried at 65 °C, and divided into branch and leaf fractions. Litter chemical quality was represented by the P, N, lignin and cellulose contents as well as the lignin:N. lignin:P, and lignin:cellulose ratios. All these variables were measured in the leaf litter fraction. The P content was determined by digestion in nitro-perchloric solution ($HNO_3/HClO_4$ 2:1 *v*/*v*) followed by colorimetric analysis at 660 nm (Embrapa 1999). The N content was determined using the Kjeldahl method (Embrapa 1997). The concentrations (%) of leaf lignin and cellulose were measured by analyzing the acid detergent fiber, as standardized by Van Soest (1967) and described by Silva (1990).

Data analysis

We used the Procrustean approach to assess the extent to which the different ecosystem compartments are related. Procrustes analysis involves minimizing the sum of the squares of the differences between corresponding coordinates (observations, species, communities, etc.) in two raw data matrices or ordination solutions (Peres-Neto and Jackson 2001). While one of the matrices remains fixed ("X": target matrix), the maximum superimposition is attained by means of adjustments of size, rotation and translation of the second matrix ("Y": rotated matrix) (Peres-Neto and Jackson 2001). After achieving the best fit, the resulting association metric is the sum of the squares of the residual differences, which is inversely proportional to the degree of relationship between the matrices (compartments in the present study).

Here we used the procrustes() function of the 'vegan' R package (Oksanen et al. 2007) to evaluate the relationship between vegetation (abundance) and the below-ground compartments (SC and SMA), and to obtain the association metric. We employed default values for all the arguments within the procrustes() function, including the scaling argument, which adjusts the matrix [Y] to have maximum similarity to matrix [X] (Cleary et al. 2012). The significance of the agreement was obtained by the permutation test with the protest() function, also available in the 'vegan' package. This function uses the relationship statistic $r = \sqrt{(1 - ss)}$, called the Procrustes( ) function, a determined number of times (permutations) to test the significance (Cleary et al. 2012).

We ran a series of simulations to evaluate the consistency of the relationship between different compartments: plant community versus SC; plant community versus SMA; and SC versus SMA . All these simulations were performed in two levels: with and without the inclusion of rare species. Non-rare species were considered to be those that occurred in more than 25 % of the plots studied (occurrence in 3 or more of the 12 plots). The resulting matrix of plant community after rare species removal was formed by 27 species (Online Resource 1). In each simulation, the relationship between vegetation and the SC and SMA compartments was investigated by considering the variation summarized on 2, 3 and 4 principal component axes. In addition, the procrustean relationship simulations were performed considering two situations: 1) relationship between [X] and [Y] compartments without removing the effect of a third compartment [Z]; 2) relationship between [X] and [Y] compartments after removed the effect of a third compartment [Z]. For this second situation we used a procedure called Partial Protest (Peres-Neto and Jackson 2001) (Fig. 2). Before these analyses, the data on abundance were Hellinger-transformed (Legendre and Gallagher 2001) and the SC and SMA matrices were Box-Cox-transformed (Legendre and Legendre 1998).

After the matrices superimpositions, a procrustean association metric, representing either the relationship between the plant community and the variation of the below-ground compartments (SC and SMA), or the relationship between SC and SMA, was extracted in the form of a numeric vector (Fig. 3). This association metric was extracted using the residuals() function, available in the 'stats' R package.

We used association metric to assess the participation of the litter quality as a link between the variations above (plant community) and below-ground (SC and SMA). To accomplish this objective, we ran a multiple

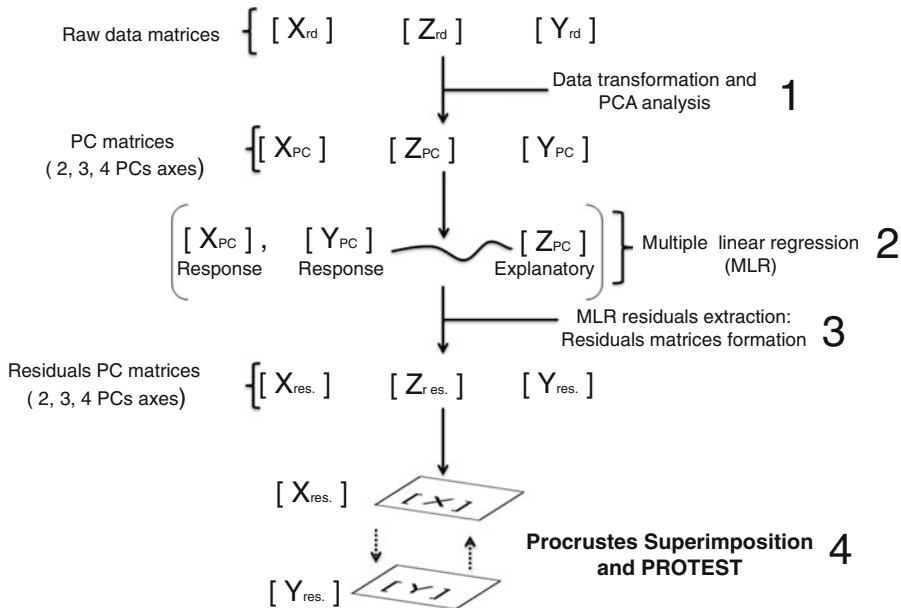

Fig. 2 Partial PROTEST. Procedure to assess the relationship between two compartments, X and Y, without the participation of a third compartment Z. 1) The matrices [Xrd], [Yrd] and [Zrd] were previously transformed and submitted to PCA. 2) Each new matrix, $[X_{PC}]$ and $[Y_{PC}]$, summarizing the variation of the compartments in the first four PCA axes, was submitted to multiple linear regression with the matrix $[Z_{PC}]$. 3) The residuals of each regression were extracted to obtain the variation in X and Y without the participation of Z: Residual PC matrices [Xres] and [Yres]. 4) Finally, these two matrices were utilized to assess the relationship and significance

linear regression having the procrustean association metrics derived from the relationships plant community versus SC, and plant community versus SMA as response variable, and the N, P, lignin and cellulose contents, and lignin:N; lignin:P, and lignin:cellulose ratios as explanatory variables (Fig. 3).

The association metric was also used to access in which of the relationships—plant community versus SC

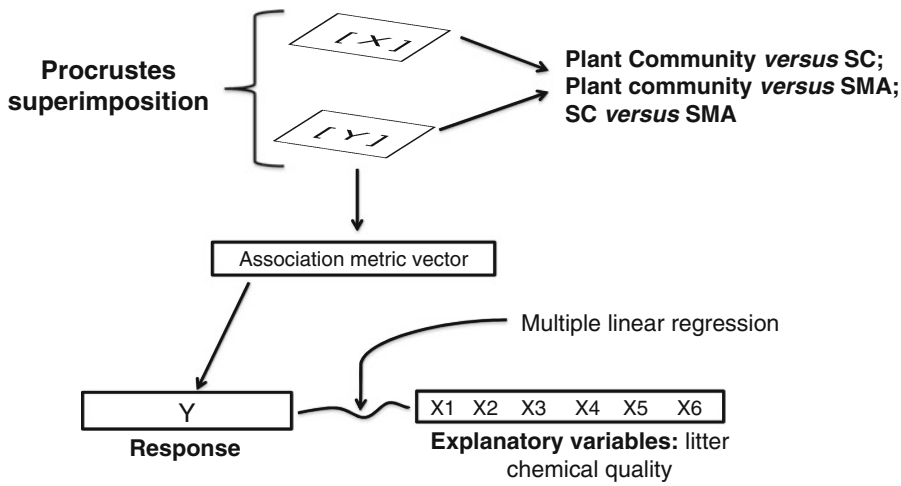

Fig. 3 Use of procrustean association metric to access the significance of litter chemical quality on the relationship between the different ecosystem compartments: plant community, soil chemistry (SC), and soil microbial activity (SMA). The association metric vector was used as a response variable, while the litter quality variables (N, P, Lignin, Cellulose, Lignin:N, Lignin:P, Lignin:Cellulose) were used as explanatory variables in multiple linear regression

and plant community versus SMA—the chemical litter quality had greater participation. This evaluation was performed in two steps: 1) extraction of the association metrics of the two relationships which were significantly affected by the litter chemical quality according to the multiple regression analyses; and 2) utilization of these metrics in means testing (*t* test, 95 % confidence).

To evaluate whether plant community and SC affected the variation in the SMA similarly or differently, we compared the means (*t test*, 95 % confidence) of the significant association metrics derived from the following relationships: plant community versus SMA and SC versus SMA. For comparison of the means, we used the agricolae R package (Mendiburu 2012).

Results

Soil chemistry (P=0.001), soil microbial activity (P=0.05) and litter quality (P=0.02) differed significantly among the studied sites (Online Resources 2). This was also indicated by an analysis of multivariate homogeneity of group dispersions, which evaluates the similarity between and within groups simultaneously (Online Resources 3).

The number of significant relationships (P<0.05) between vegetation and the below-ground variationSC and SMA was substantially greater after removing the rare species (Table 1). Only the relationship between vegetation and SMA was consistent for both situations: with and without rare species (Table 1). However, the *t* test comparing the degree of relationship between plant community and SMA in the two situations—with and without rare species—showed that this relationship was stronger without rare species (Fig 4).

The regression analyses showed that the chemical quality of the litter (independent variable) affected most of the relationships considered significant (Table 2). Both with and without rare species, the relationship between plant community and SMA was significantly influenced by the litter's chemical quality (Table 2). Litter's chemical quality had a greater influence on the response of SMA than SC to variation in the plant community (Fig. 5).

The variation in the SMA was consistently related with the variation in the SC (Table 3). However, the regression analysis between the association metrics (response) representing this relationship and the chemical quality of the litter (independent variable) did not show significant results (Table 3). The comparison between SC

Table 1 Relationship between the variation in the composition of the plant community and the variation of different below-ground compartments: soil microbial activity (SMA) and soil chemistry (SC)

[X]	[Y]	[Z]	Axes	Corr.	*P* value	Corr$^{\Delta}$.	*P* value$^{\Delta}$
Plant community	SMA	none	2	0.64	0.004	0.75	0.001
			3	0.62	0.001	0.71	0.001
			4	0.64	0.005	0.67	0.014
Plant community	SC	none	2	0.54	0.065	0.48	0.128
			3	0.49	0.221	0.66	0.004
			4	0.53	0.255	0.71	0.004
Plant community	SMA	SC	2	0.53	0.067	0.63	0.012
			3	0.54	0.128	0.54	0.103
			4	0.57	0.153	0.65	0.012
Plant community	SC	SMA	2	0.49	0.122	0.47	0.128
			3	0.48	0.238	0.56	0.061
			4	0.57	0.085	0.55	0.005

The matrices [X] and [Y] represent the superimposed compartments; [Z] represents the compartment whose effect was isolated. Relationship evaluated between compartments containing their variations summarized in 2, 3 and 4 PCA axes. Corr: correlation between compartments based on the Procrustes statistic ($r = \sqrt{(1 - ss)}$); *P*-value: significance of the Procrustes statistics based on 999 permutations. Δ: relationships evaluated without the participation of rare species

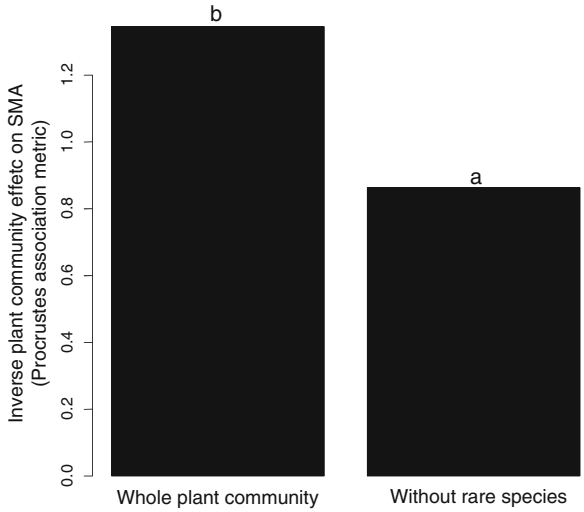

Fig. 4 Test of means (*t* test, 95 %) comparing the effect of vegetation on the variation of soil microbial activity (SMA) in two situations: with and without rare species. For the comparison, we used the association metrics representing the relationship between the plant community and the variation of the SMA (Table 1), namely: plant community with rare species vs. SMA/none (PCA axes 2, 3 and 4), and plant community without rare species vs. SMA/none (PCA axes 2, 3 and 4). The bar height is inversely proportional to the effect on the SMA

and plant community regarding their effect on the variation of SMA showed that SC had a greater influence (Fig. 6).

Discussion

To the best of our knowledge, this is the first study to utilize the Procrustean approach to test the direct relationship between different ecosystem compartments, more specifically between the plant community and the variation in the chemistry and microbial activity of the soil. Although this is an essentially correlative approach, it is reasonable to analyze the results obtained here from the perspective of the cause–effect relationship, in view of the nature of the compartments investigated. Such an analysis recognizes the importance of the soil microbial community for the composition, structure and productivity of the above-ground compartment, that is, the plant community (van der Heijden et al. 1998, 2008). Nevertheless, in studies with lesser scale of space and time like this one, the below-ground compartments more quickly respond to changes in vegetation than vice versa, with the plant community being considered a more "stable" ecosystem component than the below-ground compartments (Mitchell et al. 2010a). In this context, our results show that the variation of vegetation is consistently related to the variation in soil chemistry and microbial activity, giving support to our first hypothesis. However, this consistent relationship pattern was clearer when the simulations were conducted without

Table 2 Results of the regression analysis with the association metric between the plant community and different below-ground compartments as the response variable and the litter chemical quality as the independent variable

Related compartments		Isolated effect	Axes	*F*	*P*
[X]	[Y]	[Z]			
Plant community	SMA	none	2	$F_{6,\ 5}$=3.99	*P*=0.071
			3	$F_{6,\ 5}$=5.87	*P*=0.035
			4	$F_{6,\ 5}$=5.77	*P*=0.036
$^{\Delta}$Plant community	SMA	none	2	$F_{6,\ 5}$=7.79	*P*=0.019
			3	$F_{6,\ 5}$=0.49	*P*=0.789
			4	$F_{6,\ 5}$=8.13	*P*=0.018
$^{\Delta}$Plant community	SMA	SC	3	$F_{6,\ 5}$=4.98	*P*=0.048
			4	$F_{6,\ 5}$=6.10	*P*=0.032
$^{\Delta}$Plant community	SC	none	2	$F_{6,\ 5}$=1.44	*P*=0.350
			4	$F_{6,\ 5}$=5.24	*P*=0.044
$^{\Delta}$Plant community	SC	SMA	4	$F_{6,\ 5}$=4.82	*P*=0.052

SC soil chemistry compartment; *SMA* soil microbial activity compartment. We used association metrics of Procrustean relationships considered significant in Table 1. The symbol Δ indicates relationships investigated without the participation of rare species. The litter chemical quality variables were: percentage of leaf N, P, cellulose and the ratio of concentrations: lignin: nitrogen, lignin: phosphorous, and lignin: cellulose

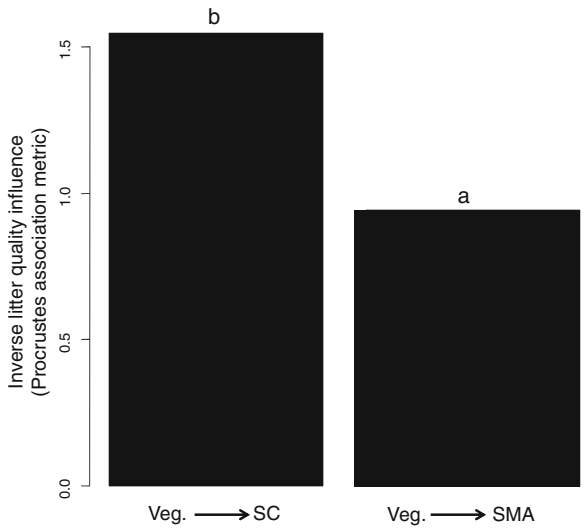

Fig. 5 Test of means (*t* test, 95 %) comparing the participation of the litter chemical quality on the effect of vegetation on the soil chemistry (SC) and soil microbial activity (SMA). For the comparison, we used the association metrics extracted from the relationships responsible for the litter quality, all without considering rare species (Table 2): plant community vs. SMA/none (axis 4) and plant community vs. SC/none (axis 4). The bar height is inversely proportional to the participation of the litter quality

considering the rare species (occurrence in less than 25 % of the plots). Indeed, there was a considerable reduction in the number of species according to this criterion, falling from 119 to 27. This result contrasts with that of Mitchell et al. (2010a), who observed that the removal of rare species did not affect the possibility of using the plant community as a predictor of the soil microbial community.

Many soil properties related to microbial activity can be affected by a reduced number of plant species or specific botanical functional groups (Scherer-Lorenzen 2008; García-Palacios et al. 2010; McLaren and Turkington 2010). Mitchell et al. (2010b), for example, observed that a single species could have significant effects on the composition and functioning of the microbial community and the soil chemistry. Corroborating that study, our results indicate that a small number of plant species are responsible to generate effects on the functioning of the ecosystem because of the more consistent effect of the plant community on the belowground variation after the removal of rare species.

The chemical quality of the plant residues incorporated in the soil is presented as one of the main factors affecting the relationship between changes in the plant community and the functioning of the microbial communities (Zak et al. 2003; Bardgett and Wardle 2010; Orwin et al. 2010; Merilä et al. 2010; Lamb et al. 2011). Our results support these findings, because besides the fact that the SMA responded more consistently to the variation in vegetation than the SC (Table 1), the response of the SMA to vegetation was also influenced more significantly by the chemical quality of the litter than was the response of the SC (Fig. 5).

The soil chemistry plays an important role in modulating the microbial functioning of the soil, as shown by various authors (Wardle 1992; Mitchell et al. 2010a,

Table 3 Relationships between the soil chemistry (SC) and variation in soil microbial activity (SMA) and their responses to the litter chemical quality

[X]	[Y]	[Z]	Axes	Corr.	*P* value	Litter quality effect
SC	SMA	none	2	0.72	0.001	$P>0.05$
			3	0.73	0.002	$P>0.05$
			4	0.71	0.001	$P>0.05$
SC	SMA	Plant community	2	0.68	0.003	$P>0.05$
			3	0.67	0.003	$P>0.05$
			4	0.74	0.001	$P>0.05$
SC	SMA	$^{\Delta}$Plant community	2	0.72	0.003	$P>0.05$
			3	0.65	0.008	$P>0.05$
			4	0.72	0.002	$P>0.05$

The matrices [X] and [Y] represent the related compartments; [Z] represents the compartment whose effect is targeted for isolation. Relationship evaluated between matrices with variation summarized in 2, 3 and 4 PCA axes. Corr: correlation between compartments based on the Procrustes statistic ($\sqrt{(1-\mathrm{ss})}$); *P*-value: significance of the Procrustes statistic based on 999 permutations. The symbol Δ indicates the plant community without the species considered rare

2012). In environments recovering from degradation, like the one of this study, where the only management was the initial one, followed by a period of time without further interventions (e.g., addition of external nutrients) and under the intense weathering of the tropical region, generates the expectation that the variation in the soil chemistry, particularly of the topmost layer (0–10 cm), would be mainly determined by the variation in the plant community. Nevertheless, our results showed that the relationship between the SC and the SMA was not driven by the chemical quality of the litter, although the SMA was consistently affected by the variation of the SC (Table 3). These results disagree with those of previous studies, which provide indications about the plant-litter-SC-SMA continuum as an indirect route for the effect of vegetation on the SMA (Mitchell et al. 2010b; Merilä et al. 2010; Lamb et al. 2011) and suggest that the plant community and soil chemistry may act independently on the soil microbial activity in our site. Therefore, it seems that recovery time of the degraded sites was not enough to reestablish the indirect relationship between the plant community and SMA through alterations in SC by litter deposition. In fact, the magnitude of the effect of the SC on the SMA was greater than the effect of the plant community on the SMA (Fig. 6), an indication that the plant community does not always serve as a proxy for the soil chemistry in terms of the effect on the SMA.

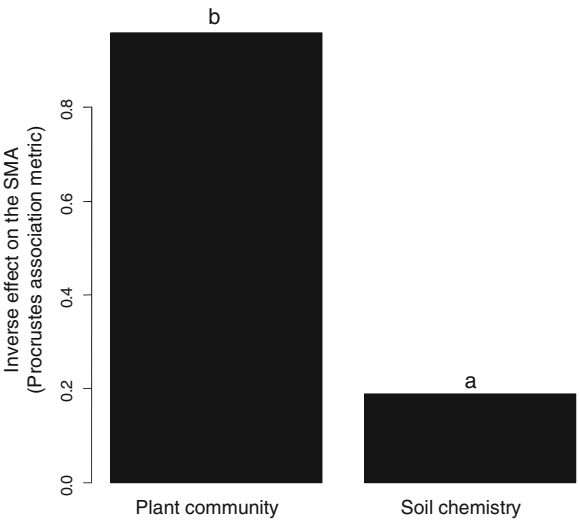

Fig. 6 Test of means (*t* test, 95 %) comparing the magnitude of the effects of plant community and soil chemistry on soil microbial activity (SMA). For the comparison, we used the association metrics extracted from the significant relationships, namely: plant community vs. SMA/SC (axes 2 and 4; Table 1) and SC vs. SMA/plant community (axes 2, 3 and 4; Table 3). The bar height is inversely proportional to the effect on the SMA

Another point to be considered is that only the aerial part of the plant community was used to characterize the chemical quality of the litter. We did not measure the chemical quality of the rhizodeposits, which have also been shown to be important to determine the microbial functioning of the soil (Berg and Smalla 2009; Chen et al. 2012). Alterations in the plant community also lead to changes in the root growth and nutrient uptake strategies, which can cause alterations in the quality and quantity of the rhizodeposits, and as a consequence, in the activity of microorganisms (Zak et al. 2003; Lamb et al. 2011). In addition to this, a recent study indicates that the variations of the above- and below-ground components of the plant community, i.e., the aerial part and the root system, are not always related to each other (Powers and Peréz-Aviles 2012). Therefore, the chemical quality of the rhizodeposits and litter could have different influences on the effect of the SC on the SMA. This is possible because the rhizodeposits provide a more readily available substrate for microbial use than the litter from the aerial parts, which take longer to become available to microbes. This time difference between deposition and consumption by the microbial community could help explain why the chemical quality of the litter was unable to influence the effect of the soil chemistry on the microbial activity.

Conclusions

We used Procrustes analysis as a tool to interrogate the causal relationships between three different compartments of a terrestrial ecosystem: plant community, soil chemistry and soil microbial activity. Our results show that the soil chemistry have a greater effect on the soil microbial activity than the plant community. In addition, the chemical quality of litter contributes significantly to the effect of the plant community on soil microbial activity, but not to the effect of soil chemistry on soil microbial activity. These findings suggest that plant community and soil chemistry act independently and that the chemical characteristics of the substrate, which were modified by its initial management, still act as a major driver of microbial activity under our study conditions. We conclude that the rupture in the in nested structure of the continuum formed

by changes in the plant community—chemical quality of litter—soil chemistry—soil microbial activity is possibly an outcome of the legacy of the chemical management of the sites.

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