Received Date: 16/09/2015

Revised Date: 11/02/2016

Accepted Date: 24/05/2016

Article Type: Articles

Running title: Soil bacterial diversity in Amazon

Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria

Teotonio Soares de Carvalho[1,2]

Ederson da Conceição Jesus[3]

Jos Barlow[2,4]

Toby A Gardner[5]

Isaac Carvalho Soares[1]

James M Tiedje[6]

Fatima Maria de Souza Moreira[1]

Authors' affiliations:

[1]Departamento de Ciência do Solo, Universidade Federal de Lavras, Lavras, Minas Gerais, 37200-000, Brazil

[2]Lancaster Environment Centre, Lancaster University, Bailrigg, Lancaster LA14YQ United Kingdom

[3]Embrapa Agrobiologia, CNPAB, Rodovia BR 465, Km 7, CEP 23891-000, Seropédica, Rio de Janeiro, Brazil
[4]Museu Paraense Emilio Goeldi, Av. Magalhães Barata, 376, Belém-Pará-Brazil
[5]Stockholm Environment Institute, Linnégatan 87D, Box 24218, Stockholm, 10451, Sweden
[6]Center for Microbial Ecology, Michigan State University, East Lansing, Michigan, 48824, USA

Corresponding author prior to publication: Teotonio Soares de Carvalho (teo.decarvalho@gmail.com)

Corresponding author after publication: Fatima Maria de Souza Moreira (fmoreira@dcs.ufla.br)

ABSTRACT

Anthropogenic pressures on tropical forests are rapidly intensifying, but our understanding of their implications for biological diversity is still very limited, especially with regard to soil biota, and in particular soil bacterial communities. Here we evaluated bacterial community composition and diversity across a gradient of land use intensity in the eastern Amazon from undisturbed primary forest, through primary forests varyingly disturbed by fire, regenerating secondary forest, pasture, and mechanized agriculture. Soil bacteria were assessed by paired-end Illumina sequencing of 16S *rRNA* gene fragments (V4 region). The resulting sequences were clustered into operational taxonomic units (OTU) at a 97% similarity threshold. Land use intensification increased the observed bacterial diversity (both OTU richness and community heterogeneity across space) and this effect was strongly associated with changes in soil pH. Moreover, land use intensification and subsequent changes in soil fertility, especially pH, altered the bacterial community composition, with pastures and areas of mechanized agriculture displaying the most contrasting communities in relation to undisturbed primary forest. Together, these results indicate that tropical forest conversion impacts soil bacteria not through loss of diversity, as previously thought, but mainly by imposing marked shifts on bacterial community composition, with unknown yet potentially important implications for ecological functions and services performed by these communities.

Keywords: *Amazon forest, below-ground biodiversity, high-throughput sequencing, drivers of bacterial community composition, 16S rRNA gene.*

INTRODUCTION

Tropical rainforests harbor some of the most biodiverse fauna and flora on the planet, and play a major role in global climate regulation and biogeochemical cycles (Malhi et al. 2014). These ecosystems are threatened by both widespread forest degradation (e.g. fire, logging, and overhunting), and clearance for agriculture and cattle production, as well as changes in temperature, precipitation and atmospheric chemistry (Malhi et al. 2014). Marked reductions in animal and plant diversity after forest conversion has been widely reported (Turner, 1996; Gibson et al., 2011; Solar et al., 2015), but the impact of deforestation on soil fauna, and in particular on soil bacterial communities, is still poorly understood, despite the key role of these organisms in the functioning of terrestrial ecosystems (Bardgett and van der Putten, 2014) and their relevance for agricultural production systems.

In the Brazilian Amazon, the conversion of forests into agricultural systems can increase the availability of mineral nutrients and reduce soil acidity (Moreira et al., 2009; Braz et al., 2013), which is expected to stimulate bacterial growth (Bardgett and Cook, 1998; Rousk et al., 2009). Furthermore, land use intensification can drive the homogenization of plant communities and vegetation structure (Arroyo-Rodríguez, 2013), possibly reducing the diversity of food resources in the rhizosphere and in the litter, the diversity of soil microhabitats, and the diversity of hosts for symbiotic bacteria (Wardle, 2006).

Intriguingly, available evidence suggests that bacteria may respond differently from other taxa, with a number of studies reporting higher alpha (local) diversity following rainforest conversion (Jesus et al., 2009; Tripathi et al., 2012; Rodrigues et al., 2013; Mendes et al., 2015). However, Rodrigues et al (2013) found that, although conversion of primary forest to pasture increases alpha diversity, it can also decrease beta diversity, possibly resulting in lower gamma (regional) diversity. Because they studied only two land uses (primary forest and pasture), their suggestion that land use intensification homogenizes bacterial communities in Amazon has yet to be tested along a comprehensive land use gradient.

This study aims to address this knowledge gap by evaluating changes in bacterial community composition and diversity in the eastern Amazon across a broad land use intensification gradient from undisturbed primary forest, through primary forests varyingly disturbed by fire, regenerating secondary forest, pasture, and mechanized agriculture. Specifically, we test the following hypotheses: (i) land use intensification homogenizes soil bacterial

communities, with increased alpha, but reduced beta and gamma diversities in more intensive land uses; (ii) soil pH and heterogeneity in soil pH are positively associated with both soil bacterial alpha and beta diversities, respectively, with the former increasing and the latter decreasing in more intensive land uses; (iii) land use intensification in the eastern Amazon changes soil bacterial community composition, including the dominance of two important phyla (*Proteobacteria* and *Acidobacteria*); and (iv) this effect is partly associated with increased nutrient availability and reduced acidity in more intensive land uses.

MATERIAL AND METHODS

Sampling

This study was conducted in the eastern Amazon, in the Santarém and Belterra municipalities in the state of Pará, Brazil (see **Figure 1** for geographical coordinates). The mean annual temperature for this area is 26 °C and the mean annual rainfall is 2,150 mm. This region was chosen because it encompasses a land use intensification gradient that is typical of many areas in the eastern Amazon, and across the human-modified tropics more generally, including the expansion of mechanized agriculture, extensive cattle production, and high rates of forest degradation from unsustainable logging and wildfires (Gardner et al., 2013). We chose the following five land-cover classes classified by Gardner et al. (2013):

- Undisturbed primary forest (UPF). Well-preserved primary forests embedded in a large sustainable use reserve, with no ground or satellite-based evidence of logging or wildfires.
- Disturbed primary forest (DPF). Primary forests recently subjected to fire as evidenced by fire scars on trees and/or by a 20-year series of satellite images (Gardner et al. 2013). We did not find any evidence of logging within this disturbance class.
- Secondary forest (SF). Areas previously deforested for agriculture, 13-20 years after abandonment.
- Pastures (Pa). Areas covered with *Brachiaria* sp. and used for cattle production.
- Mechanized agriculture (MA). Areas under intensive agriculture for production of maize, soybean, and upland rice (often in the same year).

All areas of SF, Pa, or MA were originally deforested more than 20 years ago. Although land use classification is straightforward for UPF, Pa and MA, in some cases it can be much harder to distinguish between DPF and SF: forests that have suffered multiple burns become increasingly similar to secondary forests (e.g. Barlow and Peres, 2008), and it can be difficult to detect fine-

scale spatial heterogeneity in historical patterns of forest clearance or burn intensity. As such, these two categories should be considered as broad indicators of forest disturbance and regeneration status. Details about the land use classification used in this work are provided in Gardner et al. (2013). Additional information about changes in forest structure can be found in Berenguer et al. (2014).

The sampling was conducted in April 2013, during the rainy season, in four micro-catchments selected from 18 catchments sampled as part of the Sustainable Amazon Network (Gardner et al. 2013). Four 250 m transects per land use were distributed using a stratified-random sampling design among these catchments (Figure 1; see Gardner et al., 2013, for details). The exception was MA, with five transects. At five sampling points equally spaced (50 m) along each transect, two composite soil samples (one with 50 g for molecular analyses and another with 500 g for physicochemical analysis) were taken separately by pooling three subsamples collected from the 0-10 cm depth, after removing the soil litter layer. All samples for molecular analysis were immediately placed on ice for transport and permanently stored at -80 ºC until being freeze-dried. Samples collection across all sites was completed in one week. Soil chemical and physical attributes were analyzed at the Department of Soil Science of the Federal University of Lavras, Minas Gerais, Brazil.

DNA Extraction

DNA was extracted from 0.5 g of soil using the PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc., Carlsbad, CA, USA) following the protocol suggested by Marty et al. (2012). Briefly, after adding the soil to the tubes containing the beads, a solution of sterilized skim milk 80 mg g^{-1} (Thermo Scientific™ Oxoid™ Skim Milk) was added to the soil suspension, which was heated at 70 °C for 10 min and subjected to bead-beating at maximum speed for 2 min. All subsequent steps were performed according to the manufacturer's instructions.

PCR Amplification and Sequencing

For PCR amplification, the primers 515f/806r targeting the V4 hypervariable region of the 16S *rRNA* gene were used as described in Caporaso et al. (2012). PCRs were performed in volumes of 20µL, containing 1µL of genomic DNA, 1µL of each of the forward and reverse primers (10 µM final concentration), and 17µL of the Accuprime *Pfx* Supermix (Invitrogen). Thermal cycling conditions for the PCRs were as follows: 2 min at 95 °C followed by 30 cycles of 95 °C for 20 s,

55 °C for 15 s, and 72 °C for 1 min, with a final extension at 72 °C for 1 min. The amplicons were pooled in equimolar concentration using the SequalPrep plate normalization kit (Invitrogen), and the final concentration was quantified with a Qubit 2.0 Fluorometer (Invitrogen), and the Qubit dsDNA HS Assay Kit (Invitrogen). Illumina MiSeq paired-end (2 x 150 bp) sequencing was carried out in the Research Technology Support Facility of the Michigan State University, U.S.A.

Sequence Analysis

The UPARSE pipeline (Edgar, 2013) was used for reads merging and quality filtering by truncating the sequence length at 250 bp, with a conservative maxee value of 0.25 (equivalent to one incorrect nucleotide for each of four sequences), resulting in 6 million good quality sequences from all samples. From these, 323,431 singletons (sequences occurring only once across all samples) were removed because they may result from sequencing errors and inflate diversity measures (Huse et al., 2010). The resulting sequences were dereplicated and clustered into operational taxonomic units (OTUs) at 97% similarity threshold using UPARSE, yielding 12,886 OTUs in total. Taxonomy was assigned for each representative OTU based on the Ribosomal Database Project (RDP, Release 11.3) using the RDP classifier (Wang et al., 2007) with a threshold of 0.5. Sequences classified as Chloroplast or unclassified at the domain level were removed from the downstream analysis. Archaeal sequences were kept and they comprised about 0.9% of the total number of sequences, having negligible impact on the results if removed (data not shown).

All sequences were deposited in the MG-RAST database under the ID 16339.

Diversity Analysis

Hill's number, also known as true diversity index, was used to measure alpha, beta, and gamma diversity because it integrates the most commonly used diversity indices in a single framework and it has desirable mathematical properties that make it intuitively interpretable with regards to the diversity concept (Hill, 1973; Jost, 2006). This diversity measure is defined, for $q \neq 1$, as

$$ {}^{q}D = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)} $$

in which p_i is the abundance of the i-th species in the community, S is the total number of species (OTUs in this study), and q, referred as the order of the index, determines the measure's sensitivity

to the presence of rare species. For $q = 1$, the equation above is undefined, and it is replaced by its limit as q tends to 1, as follows:

$$^{1}D = \lim_{q \to 1} {}^{q}D = \exp\left(-\sum_{i=1}^{S} p_i \log p_i \right).$$

Following the recommendation of Chao et al. (2014), we measured diversity with three values of q, namely: diversity of all OTUs (q = 0), which is equivalent to richness; "typical" OTUs (q = 1), equivalent to the exponential of the Shannon index; and dominant OTUs (q = 2), equivalent to Simpson diversity. To standardize the sampling effort, the OTU table was rarefied to the minimum sequencing depth (19,203 reads).

The variation in the identities/abundances of species among sampling units (β diversity in the sense of Whittaker (1972)) was calculated separately for each transect by dividing the total diversity observed in the transect (γ diversity) by the mean diversity across sampling points within the respective transect (α diversity) according to the multiplicative partitioning framework presented by Jost (2007). These calculations were performed for each transect separately in order to avoid conflation of beta and gamma diversity with the non-uniform spatial distribution of the sample transects (Figure 1). Consequently, in our study, beta diversity was measured as the non-directional variation in community composition (Legendre and Cáceres, 2013) within transects, i.e., across five sampling points equally distributed along 250m. The effect of land use on the calculated diversity measures was evaluated using linear models estimated by generalized least squares using the *nlme* package (Pinheiro and Bates, 2000) in R, due to heteroscedasticity, with the undisturbed primary forest as the baseline group. The independence of the residuals in those models was assumed based on the semivariograms presented in Appendix S1, as they do not indicate spatial autocorrelation among residuals. Finally, to assess the robustness of the beta diversity results to other measures of non-directional community variation, we also estimated beta diversity as total community variance for each transect, according to the method described by Legendre and Cáceres (2013).

To assess the relationship between OTU richness (response variable) and soil pH (explanatory variable), a linear mixed effects model was estimated using the *lme4* package (Bates et al., 2015) in R, with a random intercept for transects to account for the dependence among sampling points within transect. The significance of the effect of pH was assessed by the

likelihood ratio test (Zuur et al., 2009). A regression analysis was used to assess the relationship between true beta diversity (using Hill's number of order 1) and the standard deviation of soil pH for each transect.

Community Analysis

For studying the community composition, the relative abundance of each OTU was estimated by repeatedly sampling from a Dirichlet distribution as outlined by Fernandes et al. (2014). The estimated relative abundances were square-root transformed to obtain the equivalent to the Hellinger transformation, which allows the direct use of ordination methods based on Euclidean distances as shown by Legendre and Gallagher (2001). Redundancy analysis (RDA) was performed to assess the effect of each explanatory matrix (land use, soil fertility, and spatial variables) on the community composition using the *vegan* 2.3 package in R. To verify the robustness of the ordination to the relative abundance estimation, the analysis was repeated 1,000 times, resulting in practically no difference in the final ordination. The soil explanatory matrix used was based on the first two principal components calculated from the standardized soil physicochemical dataset containing the variables indicated in Appendix S3. The explanatory matrix for land use was constructed using dummy binary variables with the undisturbed primary forest as the baseline group (Borcard et al., 2011). The spatial variables were obtained through principal coordinates of neighbor matrices as described in Declerck et al (2011). Partial RDA was used to partition the community variation into the three explanatory matrices. To avoid inflation of Type I error and overestimation of the amount of explained variance, variable selection for RDA was performed by forward selection only after the significance of the global model using all explanatory variables within each explanatory matrix was verified, as proposed by Blanchet et al. (2008).

To test the effect of land use on the relative abundances of the most generalist OTUs (those occurring in more than 20% of all sites), Kruskal Wallis tests were performed using the aldex.glm function from the ALDEx2 (Fernandes et al., 2014) package in R (R Development Core Team 2013). Following the recommendation of Fernandes et al. (2014), the relative abundances were estimated by repeated samplings from a Dirichlet distribution and centered-log ratio (clr) transformed before the application of the Kruskal Wallis tests. The resulting P-values were corrected by the false discovery rate method (Benjamini and Hochberg, 1995) to avoid the inflation of Type-I error due to multiple tests.

For comparing the (log-transformed) relative abundances of *Acidobacteria*, *Proteobacteria*, and the *Proteobacteria*:*Acidobacteria* ratio among land uses, linear mixed effects models were used with random intercept for transects using the *lme4* package (Bates et al., 2015) in R. After verifying the significance of the model using likelihood ratio tests, the significance of treatment contrasts was assessed with the 'arm' package in R, using a Markov chain Monte-Carlo method (MCMC; 10,000 simulations) according to Gelman and Hill (2007).

RESULTS

Diversity and land use intensification. – Bacterial diversity was generally higher in the disturbed systems than in the undisturbed primary forest (UPF) (**Figure 2**). Based on the Hill's number (^{q}D), for all OTUs (q = 0), "typical" OTUs (q = 1), and dominant OTUs (q = 2), we observed that all components of diversity (alpha, beta, and gamma) were consistently higher in mechanized agriculture (MA) than in UPF, except for the beta diversity of dominant OTUs. All components of diversity were also higher in pasture (Pa) than in UPF, except for alpha diversity of typical OTUs and for all components of diversity for dominant OTUs. Compared to UPF, disturbed primary forest (DPF) and secondary forest (SF) showed higher gamma diversity for all OTUs, but lower alpha and gamma diversity for dominant OTUs. Beta diversity was higher in SF than in UPF for all OTUs and typical OTUs. Our results were robust to the definition and measurement of beta diversity we used here, as alternative measures produced equivalent results (Appendix S2).

Combining the results of all land uses, observed OTU richness was positively associated with soil pH ($P < 0.001$, $\chi^2(1) = 62$) (**Figure 3-A**). In the same way, beta diversity (Hill's number of order 1) for each transect was positively related to the standard deviation of soil pH in the transect, regardless the land use ($P < 0.001$, $DF_{residuals} = 19$, $F = 46$) (**Figure 3-B,** Appendix S2).

Community composition and land use intensification. – The bacterial community composition varied significantly along the gradient of land use intensity as revealed by redundancy analysis (RDA, $P < 0.001$; $F = 16.527$, $DF_{residuals} = 99$, 1000 permutations). The ordination of bacterial community composition constrained by land use showed a clear gradient of land use intensity, ranging from undisturbed primary forest to mechanized agriculture (**Figure 4**, **Figure S1-Appendix S4**). All land uses were significantly separated from one another along that gradient of community composition and land use intensity as indicated by *a posteriori* tests (**Appendix S5**).

Land use also affected the phylogenetic composition of bacterial communities, but not all land uses could be resolved from one another based on phylogenetic dissimilarities (Appendix S5).

Variance partitioning using soil variables and land use as constraining factors showed that land use explained 38% (adjusted R^2) of the total variation in community composition, while the soil variables explained 34% (adjusted R^2). The covariation between soil and land use uniquely explained 25% (adjusted R^2) of the total community variation. The projection of soil variables, especially pH, on the ordination axis resulting from RDA – using land use as constraining factor – showed that the soil variables were strongly associated with the first axis, which represented about 80% of the variance explained by land use (Figure 4). Spatial variables explained 45% (adjusted R^2) of the total community variation (RDA, $P < 0.001$, $F = 2.72$, $DF_{residuals} = 88$, 1000 permutations). However, 75% of the spatial structure of the community (variation explained by spatial variables) was explained by land use and soil variables.

For the 4,893 most generalists OTUs (those occurring in more than 20% of all sites), a significant effect of land use on abundance was observed in 3,776 (Kruskal-Wallis test; $P < 0.05$ after false discovery rate correction) (**Figure 5)**.

For the phyla *Proteobacteria* and *Acidobacteria*, we observed reduced dominance of the former in MA ($P \leq 0.001$) and Pa ($P \leq 0.05$) in relation to UPF; and increased dominance of the latter in MA ($P \leq 0.05$) in relation to UPF. The *Proteobacteria*:*Acidobacteria* ratio was significantly lower in MA ($P \leq 0.001$) and Pa ($P \leq 0.05$) than in UPF (**Figure 6-A**). At the class taxonomic level, *Alphaproteobacteria* dominated all forest systems, whereas *Acidobacteria* subdivision 6 was the dominant class in MA and *Planctomycetia* was the dominant class in Pa (**Figure 6-B**).

DISCUSSION

Diversity and land use intensification. – Microbial diversity is considered an indicator of soil health (Nielsen and Winding, 2002) because more diverse ecosystems are predicted to be more reliable (Naeem and Li, 1997; Naeem, 1998) and productive (Yachi and Loreau, 1999). However, our results suggest that the use of soil bacterial diversity in the Amazon as an indicator of soil health may be misleading because, in this study, all components of diversity (alpha, beta, and gamma) were generally higher in the more intensive land uses, especially mechanized agriculture (MA) and pasture (Pa), than in the undisturbed primary forest (UPF). These patterns of diversity were strongly influenced by the less abundant operational taxonomic units (OTUs) because as the

sensitiveness of the index to rare species was reduced (increasing the order *q*) the difference in diversity either disappeared or was inverted, except for MA (Figure 2).

Increased bacterial alpha diversity after conversion of tropical forests to anthropogenic systems has been extensively reported (Jesus et al., 2009; Tripathi et al., 2012; Rodrigues et al., 2013; Mendes et al., 2015). The clear relationship that we observed between OTU richness and soil pH indicates a likely mechanism by which land use intensification increases bacterial alpha diversity in Amazon (Figure 3a), supporting previous studies that report a linear increase in OTU richness with increasing soil pH (within the range of pH of our study, 3.7 - 7.4) in several other ecosystems (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Tripathi et al., 2012).

Tropical soils are in general highly weathered, naturally acidic, aluminum-rich, and nutrient-poor. Because these conditions are unfavorable for most crops, conversion of natural habitats to agricultural systems in the tropics is usually followed by the application of large quantities of lime (usually several tons per hectare) to neutralize the pH and reduce aluminum toxicity to plants (Sanchez, 1976; Sanchez et al., 1983). To a smaller extent, slash-and-burn practices or occasional forest fires may elevate soil pH and result in a short-term increase in soil nutrient contents in secondary and disturbed primary forests through the deposition of ashes from combusted forest biomass (Ohno and Erich, 1990; Giardina et al., 2000; Moreira et al., 2009).

Liming of acidic soils increases bacterial growth (Lupawayi et al., 2009; Rousk et al., 2009) and soil fertilization favors bacteria to the detriment of fungi, leading to a bacteria-dominated food web (Bardgett and Cook, 1998; de Vries et al., 2006). This bacterial dominance is related to increased mineralization rates (Wardle et al., 2004), increased N-losses by leaching (de Vries et al., 2006), and reduced drought resistance of the soil food web, and the processes of C and N loss they mediate (de Vries et al., 2012). In this context, further studies targeting both fungal and bacterial diversity/abundance in human-modified tropical forest landscapes are necessary to elucidate whether the commonly observed increase in local bacterial diversity after forest conversion is associated with increased bacterial dominance in these systems and, as a consequence, with the expected detrimental impacts of increased bacterial dominance on C and N retention.

While our results regarding the alpha diversity of soil bacteria are closely aligned with those of other researchers, the fact that we found higher beta diversity in more intensive land uses (Figure 2, Appendix S2) challenges the hypothesis of biotic homogenization of bacterial

communities after deforestation of tropical forests (Rodrigues et al., 2013). Those authors reported both decreased community variation (i.e. the non-directional aspect of beta diversity) and turnover in bacterial community composition (i.e. the directional aspect of beta diversity) after conversion of rainforest to pastures. They showed that, in primary forests, bacterial community dissimilarity drops quickly with increasing spatial distances (up to 10km), with that effect being observed even at small scales (i.e., distances ranging from 0.1-100 m), whereas for pastures such decrease was less pronounced. From their results, we hypothesized that at the spatial scale of the transects used in our study (250m), the heterogeneity of bacterial communities (non-directional beta diversity) would be lower in more intensive land uses than in the undisturbed primary forest, but we observed the opposite. This divergence may be due to differences in forest structure and dynamics between the two studied Amazon regions. In the western Amazon studied by Rodrigues et al. (2013), the rate of stem turnover (rate in which trees die and are replaced) is higher than in the eastern Amazon (region used in our study) partly because of differences in soil age and type of parent material (Quesada et al., 2011) and their implications on soil fertility and physical conditions (Quesada et al., 2012). It could also be that the higher intrinsic rate of natural disturbances in western Amazonian forests (Quesada et al., 2012) results in higher heterogeneity of bacterial communities in these soils when compared to those in eastern Amazonia. Taken together with the results of Lee-Cruz et al. (2013), who also found increased beta diversity of soil bacterial communities with land use intensification in Borneo (also in the humid tropics), our results indicate that rainforest conversion to agricultural systems does not necessarily cause loss of bacterial diversity through biotic homogenization.

The high levels of beta diversity we observed in soil bacteria may be related to concomitantly high levels of heterogeneity in soil pH in more intensive land uses (Figure 3b, Appendix S2). While this is not the first report of increased bacterial beta diversity after rainforest conversion (Lee-Cruz et al., 2014), it is the first indication that this result may be related to differences in soil pH heterogeneity. A recent study of bacterial diversity in grasslands reported pH and plant richness as predictors of bacterial beta diversity (mean community dissimilarity) (Prober et al., 2015). But in our study, pH heterogeneity had a remarkably dominant effect on bacterial beta diversity, in spite of the much higher plant diversity in UPF as compared to MA and Pa. Therefore, at the scale of our study, all components of diversity of soil bacteria appear to be uncoupled from plant diversity.

Community composition and land use intensification.– Supporting our last two hypotheses, we found evidence that changes in soil parameters, especially pH, mediate the effect of land use intensification on bacterial community composition, as indicated by the results of the redundancy analysis (RDA) and variance partitioning. This shift in bacterial community composition along the gradient of land use intensity, as indicated by RDA on Hellinger-transformed abundance data (Figure 4), was also observed when the same analysis was performed on Hellinger-transformed presence-absence data (Appendix S4), but the community variation explained by land use dropped from 38% (adjusted R^2) in the former to 21% (adjusted R^2) in the latter, indicating that land use intensity affects both OTUs' identities and abundances. Mechanized agriculture results in a completely distinct bacterial community in relation to that of UPF as indicated by the analysis of the most generalist OTUs (Figure 5), whereas Pa and SF have an intermediary impact, sharing both some of the OTUs most abundant in MA and some of those most abundant in UPF. Members of *Acidobacteria* subdivisions were among the dominant classes in all land uses (Figure 6) and they also were strongly related to the first ordination axis (Figure 4). In agreement with previous studies, *Acidobacteria* subdivisions 1, 2, 3, and 13 were more dominant in acidic, nutrient-poor conditions; and subgroups 4, 6, 7, and 16 were more dominant in neutral, nutrient-rich conditions (Jones et al., 2009; Lauber et al., 2009; Navarrete et al., 2013).

At the phylum level, *Acidobacteria* dominance was higher in MA than in UPF, while the dominance of *Proteobacteria* was considerably reduced in MA and Pa in relation to UPF (Figure 6). These two phyla, and their abundance ratio, have been extensively used as indicators of the soil nutritional status because *Proteobacteria* usually prefers labile organic C pools, whereas *Acidobacteria* is adapted to low organic C quality and/or quantity (Fierer et al., 2007). This ratio is usually higher in the rhizosphere or when sucrose is artificially added to soil (Fierer et al., 2007), and reduced when the vegetation is removed (Thomson et al., 2010, 2013). The observed reduction of that ratio in Pa and MA suggests that the removal of the forest cover reduces the availability of labile substrates in the soil, as previously reported for other tropical ecosystems (Islam and Weil, 2000; Solomon et al., 2007), or that other unrecognized factors are shifting this ratio in these tropical soils.

These results have implications for environmental conservation and the long-term sustainability of agricultural land-uses because, although forest disturbance and conversion to agriculture does not seem to reduce bacterial diversity, it considerably changes bacterial community composition, even in forests recovering from wildfires or agricultural abandonment.

These changes can be expected to affect ecosystem processes mediated by bacteria (Waldrop et al., 2006; McGuire et al., 2010; Kaiser et al., 2010). In fact, the metagenome predictions (Appendix S6) suggest that these changes in bacterial community composition are altering its functional traits, especially in MA, when compared to UPF, although further studies targeting functional genes or using shotgun metagenomics are necessary to confirm those predictions. Consequently, assessments of the impacts of land use intensification on tropical biota should include soil bacteria, but focus on changes in bacterial community composition and their effects on ecosystem functioning, rather than on diversity losses. Future research is also needed to verify whether the apparent favoring of soil bacteria after land use intensification in tropical forest landscapes is occurring at the detriment of soil fungi.

ACKNOWLEDGEMENTS

We are grateful to the following for financial support: Instituto Nacional de Ciência e Tecnologia – Biodiversidade e Uso da Terra na Amazônia (CNPq 574008/2008-0, and 400640/2012-0), Empresa Brasileira de Pesquisa Agropecuária – Embrapa (SEG: 02.08.06.005.00), the UK government Darwin Initiative (17-023), The Nature Conservancy, the UK Natural Environment Research Council (NERC) (NE/F01614X/1 and NE/G000816/1, NE/F015356/2; NE/l018123/1; NE/K016431/1), the Swedish Formas 2013-1571, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the research productivity scholarship and grant to F.M.S. Moreira (processes 304574/2010-4 and 477786/2011-1), the support at MSU from RDP under US Department of Energy, Grants DE-FG02-99ER62848 and DE-FC02-07ER64494, Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES; PDSE 2597/2014-04) for the scholarship provided to the first author. We also thank the farmers and workers unions of Santarém, Belterra and Paragominas and all collaborating private landowners for their support. Finally, we thank Dr. Joice Ferreira for her suggestions on the experimental design and for her support during the sampling.

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FIGURE LEGENDS

Figure 1. Satellite images of the four catchments used in this study. Each point represents one transect with five sampling units spaced by 50 meters. Point colors indicate the land use for each transect as shown in the legend. The x and y axis represent the latitude and longitude, respectively, in decimal degrees.

Figure 2. Alpha, beta and gamma bacterial diversity for all OTUs (q = 0), "typical" OTUs (q = 1), and dominant OTUs (q = 2). The bar height indicates the mean for each land use system and the error bar shows the standard error of the mean (UPF, undisturbed primary forest, n = 4; DPF, disturbed primary forest, n = 4; SF, secondary forest, n = 4; Pa, pasture, n = 4; MA, mechanized agriculture, n = 5). The means for each land use is compared to the reference (UPF), in dark grey, using generalized least squares. * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$.

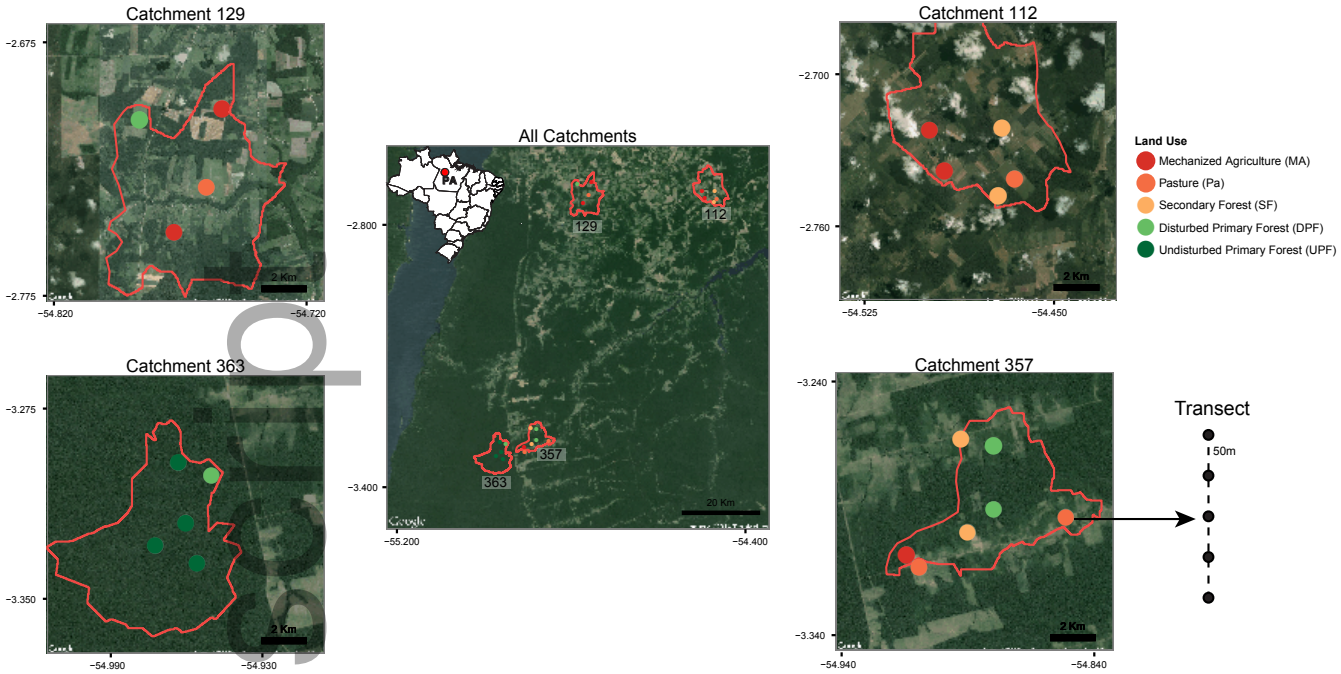
Figure 3. Models relating a) OTU richness (equivalent to Hill's number when $q = 0$) and soil pH; b) Beta diversity and standard deviation of soil pH for each transect. The fitted values for each model are represented as the black line and their standard errors are indicated by the shaded area. Both models are significant at $P < 0.001$. UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture.

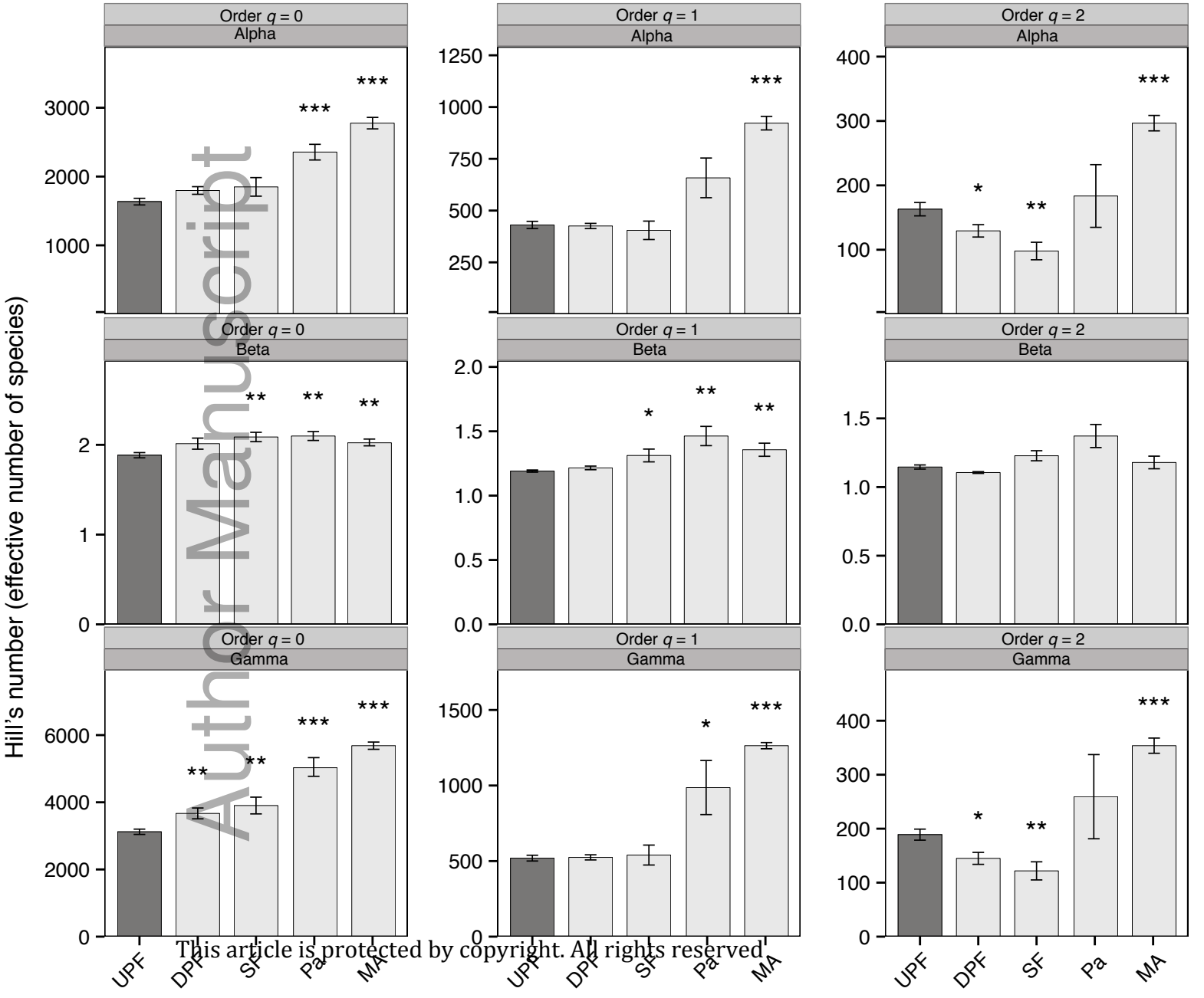
Figure 4. Redundancy analysis of the effect of land use on the abundance of soil bacteria in the eastern Amazon. Taxonomic classes (in black) and soil variables (in blue) were projected on the ordination axis using Kendall rank and Pearson correlation, respectively, and only those with significant correlation ($P \leq 0.05$; 1000 permutations) are shown. Taxonomic classes are indicated by numbers according to the legend at bottom. Point sizes indicates soil pH and their colors represent the land use system according to the legend (UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture). The percentages indicated in both axes indicate the fraction of the community variation explained by land use (38% of the total variation) that is represented by the respective axis. CEC_p, potential cation exchange capacity; CEC_e, effective cation exchange capacity.

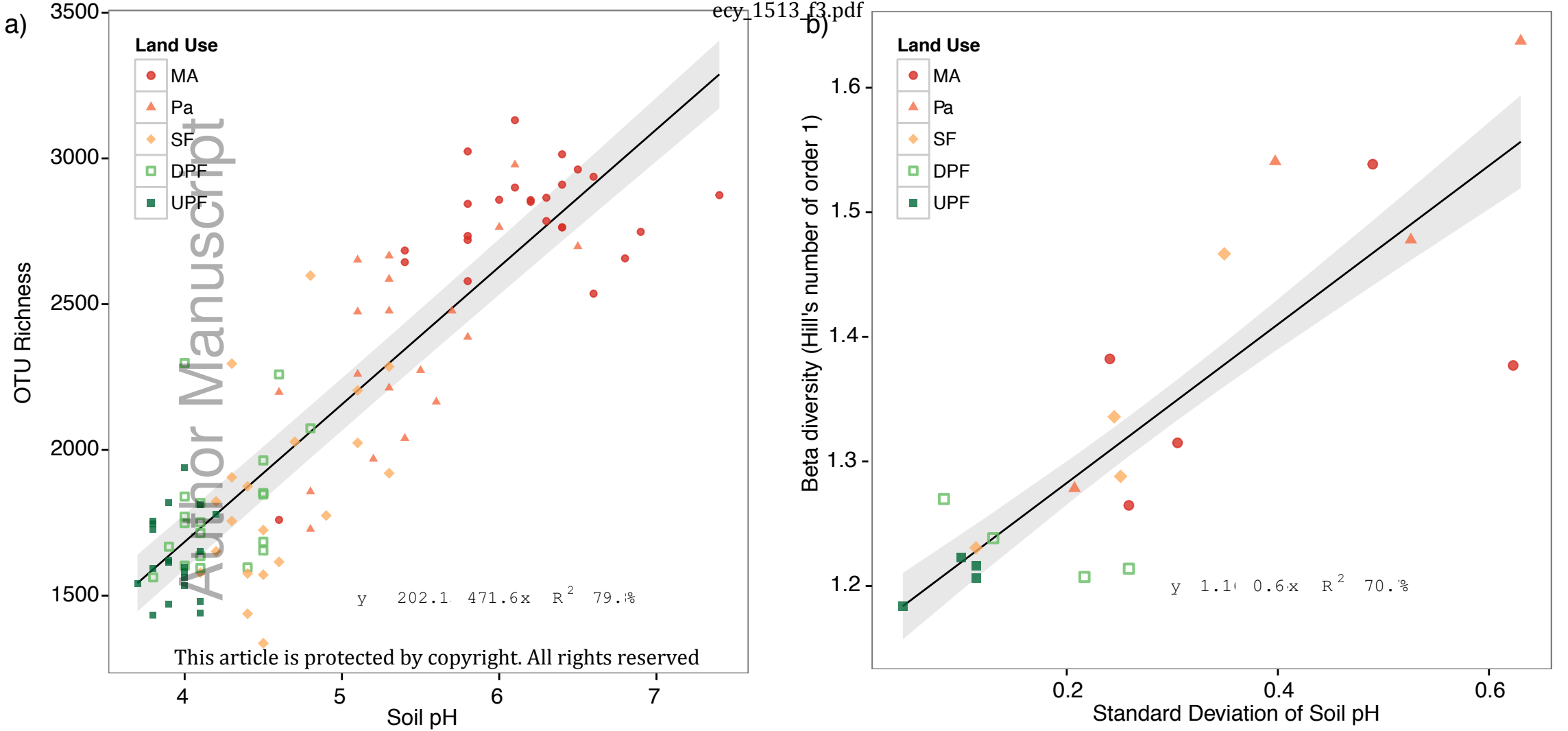
Figure 5. Heatmap of (center log-ratio) scaled relative abundances of the most generalist OTUs (occurrence > 20% for all sites). Among the 4,893 OTUs that fell into this category, only those

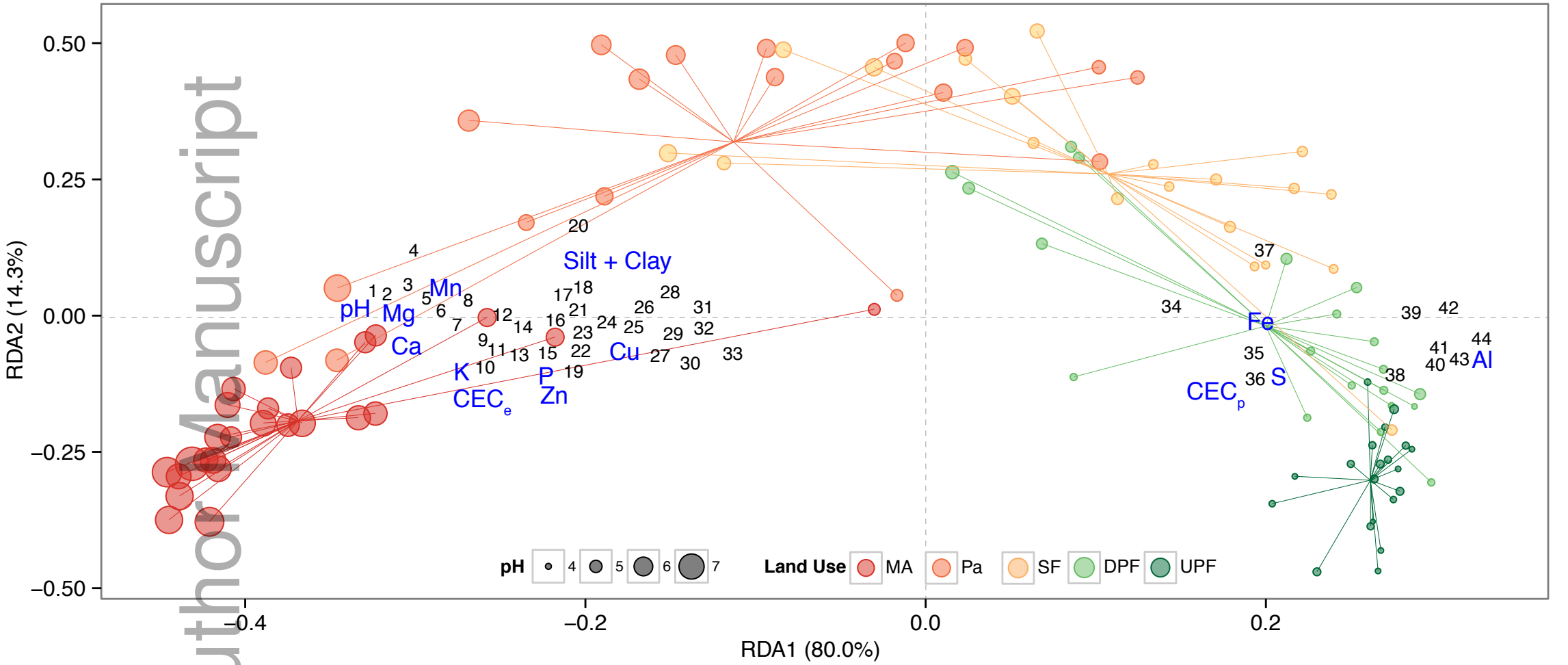
significantly affected by land use (Kruskal-Wallis test; $P < 0.05$ after false discovery correction) are shown (3,776 in total). The OTUs are arranged in rows; the samples, in columns; and the cell colors indicate the scaled relative abundance according to the legend at bottom. Rows are grouped into taxonomic classes as indicated in the right axis, and columns are grouped into land uses, as indicated at top. UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture

Figure 6. Dominant bacterial taxa in eastern Amazon soils. a) Mean relative abundances of the phylum *Proteobacteria*, *Acidobacteria*; and Protebacteria:*Acidobacteria* ratio. Vertical bars indicate the 95% confidence interval calculated using MCMC (10,000 simulations). Means are compared to UPF (in grey). * Significant at $P \leq 0.05$; *** Significant at $P \leq 0.001$. b) Relative abundances of the dominant taxonomic classes. UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture.









1 Acidobacteria G
2 Acidobacteria
3 Acidobacteria
4 Acidobacteria
5 Anaeroline
6 Betaproteobact
7 Thermoleophi
8 Acidobacteria G
9 Caldilin
10 Gemmatimonadete
11 Thermomicrob
12 Chthonomonadete
13 Nitrosp
14 Cyanobacter
15 Acidobacteria G
16 Actinobacte
17 Armatimonad
18 Flavobacte
19 Acidobacteria G
20 Bacteroidetes incerta
21 Cytophagi
22 Opitut
23 Acidobacteria G
24 Sphingobacte
25 Acidobacteria G
26 Acidobacteria G
27 Acidobacteria G
28 Methanomicrob
29 Ignavibacte
30 Chlorofle
31 Holophaga
32 Phycisphaer
33 Ardenticate
34 Acidobacteria
35 Thermoprot
36 Chlamydi
37 Planctomyce
38 Subdivisio
39 Gammaproteobacte
40 Acidobacteria
41 Acidobacteria G
42 Acidobacteria
43 Alphaproteobact
44 Acidobacteria