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

3   **bacteria**

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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.*

1   *INTRODUCTION*

2   Tropical rainforests harbor some of the most biodiverse fauna and flora on the planet, and

3   play a major role in global climate regulation and biogeochemical cycles (Malhi et al. 2014).

4   These ecosystems are threatened by both widespread forest degradation (e.g. fire, logging, and

5   overhunting), and clearance for agriculture and cattle production, as well as changes in

6   temperature, precipitation and atmospheric chemistry (Malhi et al. 2014). Marked reductions in

7   animal and plant diversity after forest conversion has been widely reported (Turner, 1996; Gibson

8   et al., 2011; Solar et al., 2015), but the impact of deforestation on soil fauna, and in particular on

9   soil bacterial communities, is still poorly understood, despite the key role of these organisms in the

10   functioning of terrestrial ecosystems (Bardgett and van der Putten, 2014) and their relevance for

11   agricultural production systems.

12   In the Brazilian Amazon, the conversion of forests into agricultural systems can increase

13   the availability of mineral nutrients and reduce soil acidity (Moreira et al., 2009; Braz et al., 2013),

14   which is expected to stimulate bacterial growth (Bardgett and Cook, 1998; Rousk et al., 2009).

15   Furthermore, land use intensification can drive the homogenization of plant communities and

16   vegetation structure (Arroyo-Rodríguez, 2013), possibly reducing the diversity of food resources

17   in the rhizosphere and in the litter, the diversity of soil microhabitats, and the diversity of hosts for

18   symbiotic bacteria (Wardle, 2006).

19   Intriguingly, available evidence suggests that bacteria may respond differently from other

20   taxa, with a number of studies reporting higher alpha (local) diversity following rainforest

21   conversion (Jesus et al., 2009; Tripathi et al., 2012; Rodrigues et al., 2013; Mendes et al., 2015).

22   However, Rodrigues et al (2013) found that, although conversion of primary forest to pasture

23   increases alpha diversity, it can also decrease beta diversity, possibly resulting in lower gamma

1   (regional) diversity. Because they studied only two land uses (primary forest and pasture), their

2   suggestion that land use intensification homogenizes bacterial communities in Amazon has yet to

3   be tested along a comprehensive land use gradient.

4   This study aims to address this knowledge gap by evaluating changes in bacterial

5   community composition and diversity in the eastern Amazon across a broad land use

6   intensification gradient from undisturbed primary forest, through primary forests varyingly

7   disturbed by fire, regenerating secondary forest, pasture, and mechanized agriculture. Specifically,

8   we test the following hypotheses: (i) land use intensification homogenizes soil bacterial

9   communities, with increased alpha, but reduced beta and gamma diversities in more intensive land

10   uses; (ii) soil pH and heterogeneity in soil pH are positively associated with both soil bacterial

11   alpha and beta diversities, respectively, with the former increasing and the latter decreasing in

12   more intensive land uses; (iii) land use intensification in the eastern Amazon changes soil bacterial

13   community composition, including the dominance of two important phyla (*Proteobacteria* and

14   *Acidobacteria*); and (iv) this effect is partly associated with increased nutrient availability and

15   reduced acidity in more intensive land uses.

16   *MATERIAL AND METHODS*

17   *Sampling*

18   This study was conducted in the eastern Amazon, in the Santarém and Belterra municipalities in

19   the state of Pará, Brazil (see **Figure 1** for geographical coordinates). The mean annual temperature

20   for this area is 26 °C and the mean annual rainfall is 2,150 mm. This region was chosen because it

21   encompasses a land use intensification gradient that is typical of many areas in the eastern

22   Amazon, and across the human-modified tropics more generally, including the expansion of

23   mechanized agriculture, extensive cattle production, and high rates of forest degradation from

1 unsustainable logging and wildfires (Gardner et al., 2013). We chose the following five land-cover

2 classes classified by Gardner et al. (2013):

3 • Undisturbed primary forest (UPF). Well-preserved primary forests embedded in a large

4 sustainable use reserve, with no ground or satellite-based evidence of logging or wildfires.

5 • Disturbed primary forest (DPF). Primary forests recently subjected to fire as evidenced by

6 fire scars on trees and/or by a 20-year series of satellite images (Gardner et al. 2013). We did

7 not find any evidence of logging within this disturbance class.

8 • Secondary forest (SF). Areas previously deforested for agriculture, 13-20 years after

9 abandonment.

10 • Pastures (Pa). Areas covered with *Brachiaria* sp. and used for cattle production.

11 • Mechanized agriculture (MA). Areas under intensive agriculture for production of maize,

12 soybean, and upland rice (often in the same year).

13 All areas of SF, Pa, or MA were originally deforested more than 20 years ago. Although

14 land use classification is straightforward for UPF, Pa and MA, in some cases it can be much harder

15 to distinguish between DPF and SF: forests that have suffered multiple burns become increasingly

16 similar to secondary forests (e.g. Barlow and Peres, 2008), and it can be difficult to detect fine-

17 scale spatial heterogeneity in historical patterns of forest clearance or burn intensity. As such,

18 these two categories should be considered as broad indicators of forest disturbance and

19 regeneration status. Details about the land use classification used in this work are provided in

20 Gardner et al. (2013). Additional information about changes in forest structure can be found in

21 Berenguer et al. (2014).

22 The sampling was conducted in April 2013, during the rainy season, in four micro-

23 catchments selected from 18 catchments sampled as part of the Sustainable Amazon Network

24 (Gardner et al. 2013). Four 250 m transects per land use were distributed using a stratified-random

1   sampling design among these catchments (Figure 1; see Gardner et al., 2013, for details). The

2   exception was MA, with five transects. At five sampling points equally spaced (50 m) along each

3   transect, two composite soil samples (one with 50 g for molecular analyses and another with 500 g

4   for physicochemical analysis) were taken separately by pooling three subsamples collected from

5   the 0-10 cm depth, after removing the soil litter layer. All samples for molecular analysis were

6   immediately placed on ice for transport and permanently stored at -80 ºC until being freeze-dried.

7   Samples collection across all sites was completed in one week. Soil chemical and physical

8   attributes were analyzed at the Department of Soil Science of the Federal University of Lavras,

9   Minas Gerais, Brazil.

10

11   *DNA Extraction*

12   DNA was extracted from 0.5 g of soil using the PowerSoil DNA Isolation Kit (MO BIO

13   Laboratories Inc., Carlsbad, CA, USA) following the protocol suggested by Marty et al. (2012).

14   Briefly, after adding the soil to the tubes containing the beads, a solution of sterilized skim milk 80

15   mg g^{-1} (Thermo Scientific™ Oxoid™ Skim Milk) was added to the soil suspension, which was

16   heated at 70 °C for 10 min and subjected to bead-beating at maximum speed for 2 min. All

17   subsequent steps were performed according to the manufacturer's instructions.

18

19   *PCR Amplification and Sequencing*

20   For PCR amplification, the primers 515f/806r targeting the V4 hypervariable region of the 16S

21   *rRNA* gene were used as described in Caporaso et al. (2012). PCRs were performed in volumes of

22   20µL, containing 1µL of genomic DNA, 1µL of each of the forward and reverse primers (10 µM

23   final concentration), and 17µL of the Accuprime *Pfx* Supermix (Invitrogen). Thermal cycling

24   conditions for the PCRs were as follows: 2 min at 95 °C followed by 30 cycles of 95 °C for 20 s,

1   55 °C for 15 s, and 72 °C for 1 min, with a final extension at 72 °C for 1 min. The amplicons were

2   pooled in equimolar concentration using the SequalPrep plate normalization kit (Invitrogen), and

3   the final concentration was quantified with a Qubit 2.0 Fluorometer (Invitrogen), and the Qubit

4   dsDNA HS Assay Kit (Invitrogen). Illumina MiSeq paired-end (2 x 150 bp) sequencing was

5   carried out in the Research Technology Support Facility of the Michigan State University, U.S.A.

6

7   *Sequence Analysis*

8   The UPARSE pipeline (Edgar, 2013) was used for reads merging and quality filtering by

9   truncating the sequence length at 250 bp, with a conservative maxee value of 0.25 (equivalent to

10   one incorrect nucleotide for each of four sequences), resulting in 6 million good quality sequences

11   from all samples. From these, 323,431 singletons (sequences occurring only once across all

12   samples) were removed because they may result from sequencing errors and inflate diversity

13   measures (Huse et al., 2010). The resulting sequences were dereplicated and clustered into

14   operational taxonomic units (OTUs) at 97% similarity threshold using UPARSE, yielding 12,886

15   OTUs in total. Taxonomy was assigned for each representative OTU based on the Ribosomal

16   Database Project (RDP, Release 11.3) using the RDP classifier (Wang et al., 2007) with a

17   threshold of 0.5. Sequences classified as Chloroplast or unclassified at the domain level were

18   removed from the downstream analysis. Archaeal sequences were kept and they comprised about

19   0.9% of the total number of sequences, having negligible impact on the results if removed (data

20   not shown).

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

22

23   *Diversity Analysis*

1   Hill's number, also known as true diversity index, was used to measure alpha, beta, and gamma

2   diversity because it integrates the most commonly used diversity indices in a single framework and

3   it has desirable mathematical properties that make it intuitively interpretable with regards to the

4   diversity concept (Hill, 1973; Jost, 2006). This diversity measure is defined, for $q \neq 1$, as

5
$$ {}^{q}D = \left(\sum_{i=1}^{S} p_i^q \right)^{1/(1-q)} $$

6   in which p_i is the abundance of the i-th species in the community, S is the total number of species

7   (OTUs in this study), and q, referred as the order of the index, determines the measure's sensitivity

8   to the presence of rare species. For $q = 1$, the equation above is undefined, and it is replaced by its

9   limit as q tends to 1, as follows:

10
$$ {}^{1}D = \lim_{q \to 1} {}^{q}D = \exp\left(-\sum_{i=1}^{S} p_i \log p_i \right). $$

11

12   Following the recommendation of Chao et al. (2014), we measured diversity with three

13   values of q, namely: diversity of all OTUs (q = 0), which is equivalent to richness; "typical" OTUs

14   (q = 1), equivalent to the exponential of the Shannon index; and dominant OTUs (q = 2),

15   equivalent to Simpson diversity. To standardize the sampling effort, the OTU table was rarefied to

16   the minimum sequencing depth (19,203 reads).

17   The variation in the identities/abundances of species among sampling units (β diversity in

18   the sense of Whittaker (1972)) was calculated separately for each transect by dividing the total

19   diversity observed in the transect (γ diversity) by the mean diversity across sampling points within

20   the respective transect (α diversity) according to the multiplicative partitioning framework

21   presented by Jost (2007). These calculations were performed for each transect separately in order

22   to avoid conflation of beta and gamma diversity with the non-uniform spatial distribution of the

1   sample transects (Figure 1). Consequently, in our study, beta diversity was measured as the non-

2   directional variation in community composition (Legendre and Cáceres, 2013) within transects,

3   i.e., across five sampling points equally distributed along 250m. The effect of land use on the

4   calculated diversity measures was evaluated using linear models estimated by generalized least

5   squares using the *nlme* package (Pinheiro and Bates, 2000) in R, due to heteroscedasticity, with the

6   undisturbed primary forest as the baseline group. The independence of the residuals in those

7   models was assumed based on the semivariograms presented in Appendix S1, as they do not

8   indicate spatial autocorrelation among residuals. Finally, to assess the robustness of the beta

9   diversity results to other measures of non-directional community variation, we also estimated beta

10 diversity as total community variance for each transect, according to the method described by

11 Legendre and Cáceres (2013).

12   To assess the relationship between OTU richness (response variable) and soil pH

13 (explanatory variable), a linear mixed effects model was estimated using the *lme4* package (Bates

14 et al., 2015) in R, with a random intercept for transects to account for the dependence among

15 sampling points within transect. The significance of the effect of pH was assessed by the

16 likelihood ratio test (Zuur et al., 2009). A regression analysis was used to assess the relationship

17 between true beta diversity (using Hill's number of order 1) and the standard deviation of soil pH

18 for each transect.

19

20 *Community Analysis*

21 For studying the community composition, the relative abundance of each OTU was estimated by

22 repeatedly sampling from a Dirichlet distribution as outlined by Fernandes et al. (2014). The

23 estimated relative abundances were square-root transformed to obtain the equivalent to the

24 Hellinger transformation, which allows the direct use of ordination methods based on Euclidean

1   distances as shown by Legendre and Gallagher (2001). Redundancy analysis (RDA) was

2   performed to assess the effect of each explanatory matrix (land use, soil fertility, and spatial

3   variables) on the community composition using the *vegan* 2.3 package in R. To verify the

4   robustness of the ordination to the relative abundance estimation, the analysis was repeated 1,000

5   times, resulting in practically no difference in the final ordination. The soil explanatory matrix

6   used was based on the first two principal components calculated from the standardized soil

7   physicochemical dataset containing the variables indicated in Appendix S3. The explanatory

8   matrix for land use was constructed using dummy binary variables with the undisturbed primary

9   forest as the baseline group (Borcard et al., 2011). The spatial variables were obtained through

10   principal coordinates of neighbor matrices as described in Declerck et al (2011). Partial RDA was

11   used to partition the community variation into the three explanatory matrices. To avoid inflation of

12   Type I error and overestimation of the amount of explained variance, variable selection for RDA

13   was performed by forward selection only after the significance of the global model using all

14   explanatory variables within each explanatory matrix was verified, as proposed by Blanchet et al.

15   (2008).

16   To test the effect of land use on the relative abundances of the most generalist OTUs (those

17   occurring in more than 20% of all sites), Kruskal Wallis tests were performed using the aldex.glm

18   function from the ALDEx2 (Fernandes et al., 2014) package in R (R Development Core Team

19   2013). Following the recommendation of Fernandes et al. (2014), the relative abundances were

20   estimated by repeated samplings from a Dirichlet distribution and centered-log ratio (clr)

21   transformed before the application of the Kruskal Wallis tests. The resulting P-values were

22   corrected by the false discovery rate method (Benjamini and Hochberg, 1995) to avoid the

23   inflation of Type-I error due to multiple tests.

1   For comparing the (log-transformed) relative abundances of *Acidobacteria*,

2   *Proteobacteria*, and the *Proteobacteria:Acidobacteria* ratio among land uses, linear mixed effects

3   models were used with random intercept for transects using the *lme4* package (Bates et al., 2015)

4   in R. After verifying the significance of the model using likelihood ratio tests, the significance of

5   treatment contrasts was assessed with the 'arm' package in R, using a Markov chain Monte-Carlo

6   method (MCMC; 10,000 simulations) according to Gelman and Hill (2007).

7   *RESULTS*

8   *Diversity and land use intensification.* – Bacterial diversity was generally higher in the

9   disturbed systems than in the undisturbed primary forest (UPF) (**Figure 2**). Based on the Hill's

10   number (^qD), for all OTUs (q = 0), "typical" OTUs (q = 1), and dominant OTUs (q = 2), we

11   observed that all components of diversity (alpha, beta, and gamma) were consistently higher in

12   mechanized agriculture (MA) than in UPF, except for the beta diversity of dominant OTUs. All

13   components of diversity were also higher in pasture (Pa) than in UPF, except for alpha diversity of

14   typical OTUs and for all components of diversity for dominant OTUs. Compared to UPF,

15   disturbed primary forest (DPF) and secondary forest (SF) showed higher gamma diversity for all

16   OTUs, but lower alpha and gamma diversity for dominant OTUs. Beta diversity was higher in SF

17   than in UPF for all OTUs and typical OTUs. Our results were robust to the definition and

18   measurement of beta diversity we used here, as alternative measures produced equivalent results

19   (Appendix S2).

20   Combining the results of all land uses, observed OTU richness was positively associated

21   with soil pH ($P < 0.001$, $\chi^2(1) = 62$) (**Figure 3-A**). In the same way, beta diversity (Hill's number

22   of order 1) for each transect was positively related to the standard deviation of soil pH in the

23   transect, regardless the land use ($P < 0.001$, $DF_{residuals} = 19$, $F = 46$) (**Figure 3-B,** Appendix S2).

1 *Community composition and land use intensification.* – The bacterial community composition

2 varied significantly along the gradient of land use intensity as revealed by redundancy analysis

3 (RDA, P < 0.001; $F = 16.527$, $DF_{residuals} = 99$, 1000 permutations). The ordination of bacterial

4 community composition constrained by land use showed a clear gradient of land use intensity,

5 ranging from undisturbed primary forest to mechanized agriculture (**Figure 4**, **Figure S1-**

6 **Appendix S4**). All land uses were significantly separated from one another along that gradient of

7 community composition and land use intensity as indicated by *a posteriori* tests (**Appendix S5**).

8 Land use also affected the phylogenetic composition of bacterial communities, but not all land

9 uses could be resolved from one another based on phylogenetic dissimilarities (Appendix S5).

10   Variance partitioning using soil variables and land use as constraining factors showed that

11 land use explained 38% (adjusted R^2) of the total variation in community composition, while the

12 soil variables explained 34% (adjusted R^2). The covariation between soil and land use uniquely

13 explained 25% (adjusted R^2) of the total community variation. The projection of soil variables,

14 especially pH, on the ordination axis resulting from RDA – using land use as constraining factor –

15 showed that the soil variables were strongly associated with the first axis, which represented about

16 80% of the variance explained by land use (Figure 4). Spatial variables explained 45% (adjusted

17 R^2) of the total community variation (RDA, $P < 0.001$, $F = 2.72$, $DF_{residuals} = 88$, 1000

18 permutations). However, 75% of the spatial structure of the community (variation explained by

19 spatial variables) was explained by land use and soil variables.

20   For the 4,893 most generalists OTUs (those occurring in more than 20% of all sites), a

21 significant effect of land use on abundance was observed in 3,776 (Kruskal-Wallis test; $P < 0.05$

22 after false discovery rate correction) (**Figure 5**).

23   For the phyla *Proteobacteria* and *Acidobacteria*, we observed reduced dominance of the

24 former in MA ($P \leq 0.001$) and Pa ($P \leq 0.05$) in relation to UPF; and increased dominance of the

1 latter in MA ($P \leq 0.05$) in relation to UPF. The *Proteobacteria:Acidobacteria* ratio was

2 significantly lower in MA ($P \leq 0.001$) and Pa ($P \leq 0.05$) than in UPF (**Figure 6-A**). At the class

3 taxonomic level, *Alphaproteobacteria* dominated all forest systems, whereas *Acidobacteria*

4 subdivision 6 was the dominant class in MA and *Planctomycetia* was the dominant class in Pa

5 (**Figure 6-B**).

6 *DISCUSSION*

7 *Diversity and land use intensification.* – Microbial diversity is considered an indicator of soil

8 health (Nielsen and Winding, 2002) because more diverse ecosystems are predicted to be more

9 reliable (Naeem and Li, 1997; Naeem, 1998) and productive (Yachi and Loreau, 1999). However,

10 our results suggest that the use of soil bacterial diversity in the Amazon as an indicator of soil

11 health may be misleading because, in this study, all components of diversity (alpha, beta, and

12 gamma) were generally higher in the more intensive land uses, especially mechanized agriculture

13 (MA) and pasture (Pa), than in the undisturbed primary forest (UPF). These patterns of diversity

14 were strongly influenced by the less abundant operational taxonomic units (OTUs) because as the

15 sensitiveness of the index to rare species was reduced (increasing the order *q*) the difference in

16 diversity either disappeared or was inverted, except for MA (Figure 2).

17 Increased bacterial alpha diversity after conversion of tropical forests to anthropogenic

18 systems has been extensively reported (Jesus et al., 2009; Tripathi et al., 2012; Rodrigues et al.,

19 2013; Mendes et al., 2015). The clear relationship that we observed between OTU richness and

20 soil pH indicates a likely mechanism by which land use intensification increases bacterial alpha

21 diversity in Amazon (Figure 3a), supporting previous studies that report a linear increase in OTU

22 richness with increasing soil pH (within the range of pH of our study, 3.7 - 7.4) in several other

1   ecosystems (Fierer and Jackson, 2006; Lauber et al., 2009; Rousk et al., 2010; Tripathi et al.,

2   2012).

3   Tropical soils are in general highly weathered, naturally acidic, aluminum-rich, and

4   nutrient-poor. Because these conditions are unfavorable for most crops, conversion of natural

5   habitats to agricultural systems in the tropics is usually followed by the application of large

6   quantities of lime (usually several tons per hectare) to neutralize the pH and reduce aluminum

7   toxicity to plants (Sanchez, 1976; Sanchez et al., 1983). To a smaller extent, slash-and-burn

8   practices or occasional forest fires may elevate soil pH and result in a short-term increase in soil

9   nutrient contents in secondary and disturbed primary forests through the deposition of ashes from

10   combusted forest biomass (Ohno and Erich, 1990; Giardina et al., 2000; Moreira et al., 2009).

11   Liming of acidic soils increases bacterial growth (Lupawayi et al., 2009; Rousk et al.,

12   2009) and soil fertilization favors bacteria to the detriment of fungi, leading to a bacteria-

13   dominated food web (Bardgett and Cook, 1998; de Vries et al., 2006). This bacterial dominance is

14   related to increased mineralization rates (Wardle et al., 2004), increased N-losses by leaching (de

15   Vries et al., 2006), and reduced drought resistance of the soil food web, and the processes of C and

16   N loss they mediate (de Vries et al., 2012). In this context, further studies targeting both fungal

17   and bacterial diversity/abundance in human-modified tropical forest landscapes are necessary to

18   elucidate whether the commonly observed increase in local bacterial diversity after forest

19   conversion is associated with increased bacterial dominance in these systems and, as a

20   consequence, with the expected detrimental impacts of increased bacterial dominance on C and N

21   retention.

22   While our results regarding the alpha diversity of soil bacteria are closely aligned with

23   those of other researchers, the fact that we found higher beta diversity in more intensive land uses

24   (Figure 2, Appendix S2) challenges the hypothesis of biotic homogenization of bacterial

1   communities after deforestation of tropical forests (Rodrigues et al., 2013). Those authors reported

2   both decreased community variation (i.e. the non-directional aspect of beta diversity) and turnover

3   in bacterial community composition (i.e. the directional aspect of beta diversity) after conversion

4   of rainforest to pastures. They showed that, in primary forests, bacterial community dissimilarity

5   drops quickly with increasing spatial distances (up to 10km), with that effect being observed even

6   at small scales (i.e., distances ranging from 0.1-100 m), whereas for pastures such decrease was

7   less pronounced. From their results, we hypothesized that at the spatial scale of the transects used

8   in our study (250m), the heterogeneity of bacterial communities (non-directional beta diversity)

9   would be lower in more intensive land uses than in the undisturbed primary forest, but we

10   observed the opposite. This divergence may be due to differences in forest structure and dynamics

11   between the two studied Amazon regions. In the western Amazon studied by Rodrigues et al.

12   (2013), the rate of stem turnover (rate in which trees die and are replaced) is higher than in the

13   eastern Amazon (region used in our study) partly because of differences in soil age and type of

14   parent material (Quesada et al., 2011) and their implications on soil fertility and physical

15   conditions (Quesada et al., 2012). It could also be that the higher intrinsic rate of natural

16   disturbances in western Amazonian forests (Quesada et al., 2012) results in higher heterogeneity

17   of bacterial communities in these soils when compared to those in eastern Amazonia. Taken

18   together with the results of Lee-Cruz et al. (2013), who also found increased beta diversity of soil

19   bacterial communities with land use intensification in Borneo (also in the humid tropics), our

20   results indicate that rainforest conversion to agricultural systems does not necessarily cause loss of

21   bacterial diversity through biotic homogenization.

22   The high levels of beta diversity we observed in soil bacteria may be related to

23   concomitantly high levels of heterogeneity in soil pH in more intensive land uses (Figure 3b,

24   Appendix S2). While this is not the first report of increased bacterial beta diversity after rainforest

1 conversion (Lee-Cruz et al., 2014), it is the first indication that this result may be related to

2 differences in soil pH heterogeneity. A recent study of bacterial diversity in grasslands reported

3 pH and plant richness as predictors of bacterial beta diversity (mean community dissimilarity)

4 (Prober et al., 2015). But in our study, pH heterogeneity had a remarkably dominant effect on

5 bacterial beta diversity, in spite of the much higher plant diversity in UPF as compared to MA and

6 Pa. Therefore, at the scale of our study, all components of diversity of soil bacteria appear to be

7 uncoupled from plant diversity.

8 *Community composition and land use intensification.*– Supporting our last two hypotheses, we

9 found evidence that changes in soil parameters, especially pH, mediate the effect of land use

10 intensification on bacterial community composition, as indicated by the results of the redundancy

11 analysis (RDA) and variance partitioning. This shift in bacterial community composition along the

12 gradient of land use intensity, as indicated by RDA on Hellinger-transformed abundance data

13 (Figure 4), was also observed when the same analysis was performed on Hellinger-transformed

14 presence-absence data (Appendix S4), but the community variation explained by land use dropped

15 from 38% (adjusted R^2) in the former to 21% (adjusted R^2) in the latter, indicating that land use

16 intensity affects both OTUs' identities and abundances. Mechanized agriculture results in a

17 completely distinct bacterial community in relation to that of UPF as indicated by the analysis of

18 the most generalist OTUs (Figure 5), whereas Pa and SF have an intermediary impact, sharing

19 both some of the OTUs most abundant in MA and some of those most abundant in UPF. Members

20 of *Acidobacteria* subdivisions were among the dominant classes in all land uses (Figure 6) and

21 they also were strongly related to the first ordination axis (Figure 4). In agreement with previous

22 studies, *Acidobacteria* subdivisions 1, 2, 3, and 13 were more dominant in acidic, nutrient-poor

23 conditions; and subgroups 4, 6, 7, and 16 were more dominant in neutral, nutrient-rich conditions

24 (Jones et al., 2009; Lauber et al., 2009; Navarrete et al., 2013).

16

1   At the phylum level, *Acidobacteria* dominance was higher in MA than in UPF, while the

2   dominance of *Proteobacteria* was considerably reduced in MA and Pa in relation to UPF (Figure

3   6). These two phyla, and their abundance ratio, have been extensively used as indicators of the soil

4   nutritional status because *Proteobacteria* usually prefers labile organic C pools, whereas

5   *Acidobacteria* is adapted to low organic C quality and/or quantity (Fierer et al., 2007). This ratio is

6   usually higher in the rhizosphere or when sucrose is artificially added to soil (Fierer et al., 2007),

7   and reduced when the vegetation is removed (Thomson et al., 2010, 2013). The observed reduction

8   of that ratio in Pa and MA suggests that the removal of the forest cover reduces the availability of

9   labile substrates in the soil, as previously reported for other tropical ecosystems (Islam and Weil,

10   2000; Solomon et al., 2007), or that other unrecognized factors are shifting this ratio in these

11   tropical soils.

12   These results have implications for environmental conservation and the long-term

13   sustainability of agricultural land-uses because, although forest disturbance and conversion to

14   agriculture does not seem to reduce bacterial diversity, it considerably changes bacterial

15   community composition, even in forests recovering from wildfires or agricultural abandonment.

16   These changes can be expected to affect ecosystem processes mediated by bacteria (Waldrop et al.,

17   2006; McGuire et al., 2010; Kaiser et al., 2010). In fact, the metagenome predictions (Appendix

18   S6) suggest that these changes in bacterial community composition are altering its functional traits,

19   especially in MA, when compared to UPF, although further studies targeting functional genes or

20   using shotgun metagenomics are necessary to confirm those predictions. Consequently,

21   assessments of the impacts of land use intensification on tropical biota should include soil bacteria,

22   but focus on changes in bacterial community composition and their effects on ecosystem

23   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

1 *LITERATURE CITED*

2 Arroyo-Rodríguez, V., M. Rös, F. Escobar, F. P. L. Melo, B. A. Santos, M. Tabarelli, and R. Chazdon.

3   2013. Plant β-diversity in fragmented rain forests: Testing floristic homogenization and

4   differentiation hypotheses. Journal of Ecology **101**:1449-1458.

5 Bardgett, R. D., and R. Cook. 1998. Functional aspects of soil animal diversity in agricultural grasslands.

6   Applied Soil Ecology **10**:263-276.

7 Bardgett, R. D., and W. H. van der Putten. 2014. Belowground biodiversity and ecosystem functioning.

8   Nature **515**:505-511.

9 Barlow, J., and C. A. Peres. 2008. Fire-mediated dieback and compositional cascade in an Amazonian

10   forest. Philosophical transactions of the Royal Society of London. Series B, Biological sciences

11   **363**:1787-1794.

12 Bates, D., Machler, M., Bolker, B., and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using

13   lme4. Journal of Statistical Software **67**:1-48.

14 Benjamini, Y., and Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful

15   approach to multiple testing. Journal of the Royal Statistical Society Series B **57**:289–300.

16 Berenguer, E., J. Ferreira, T. A. Gardner, L. E. O. C. Aragão, P. B. De Camargo, C. E. Cerri, M. Durigan,

17   R. C. D. Oliveira, I. C. G. Vieira, and J. Barlow. 2014. A large-scale field assessment of carbon

18   stocks in human-modified tropical forests. Global Change Biology **2005**:3713-3726.

19 Blanchet, F. G., Legendre, P., and D. Borcard. 2008. Forward selection of explanatory variables. Ecology

20   **89**:2623-2632.

21 Borcard, D., Gillet, F., and P. Legendre. 2011. Numerical Ecology with R. Springer. New York, NY.

22 Caporaso, J. G., et al. 2012. Ultra-high-throughput microbial community analysis on the Illumina HiSeq

23   and MiSeq platforms. The ISME Journal **6**:1621-1624.

1   Chao, A., N. J. Gotelli, and T. C. Hsieh. 2014. Rarefaction and extrapolation with Hill numbers : a

2   framework for sampling and estimation in species diversity studies. Ecological Monographs

3   **84**:45-67.

4   De Souza Braz, A. M., A. R. Fernandes, and L. R. F. Alleoni. 2013. Soil attributes after the conversion

5   from forest to pasture in Amazon. Land Degradation and Development **24**:33-38.

6   de Vries, F. T., J. Bloem, H. Quirk, C. J. Stevens, R. Bol, and R. D. Bardgett. 2012. Extensive

7   Management Promotes Plant and Microbial Nitrogen Retention in Temperate Grassland. PLoS

8   ONE **7**:1-12.

9   de Vries, F. T., E. Hoffland, N. van Eekeren, L. Brussaard, and J. Bloem. 2006. Fungal/bacterial ratios in

10   grasslands with contrasting nitrogen management. Soil Biology and Biochemistry **38**:2092-2103.

11   Declerck, S. A. J., J. S. Coronel, P. Legendre, and L. Brendonck. 2011. Scale dependency of processes

12   structuring metacommunities of cladocerans in temporary pools of High-Andes wetlands.

13   Ecography **34**:296-305.

14   Edgar, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature

15   methods **10**:996-998.

16   Fernandes, A. D., J. N. Reid, J. M. Macklaim, T. A. McMurrough, D. R. Edgell, and G. B. Gloor. 2014.

17   Unifying the analysis of high-throughput sequencing datasets: characterizing RNA-seq, 16S rRNA

18   gene sequencing and selective growth experiments by compositional data analysis. Microbiome

19   **2**:15-15.

20   Fierer, N., M. A. Bradford, and R. B. Jackson. 2007. Toward an ecological classification of soil bacteria.

21   Ecology **88**:1354-1364.

22   Fierer, N., and R. Jackson. 2006. The diversity and biogeography of soil bacterial communities.

23   Proceedings of the National Academy of Sciences of the United States of America **103**:626-631.

24   Gardner, T. A., et al. 2013. A social and ecological assessment of tropical land uses at multiple scales : the

Sustainable Amazon Network. Philosophical transactions of the Royal Society of London. Series B, Biological sciences **368**:20120166-20120166.

Gelman, A., and J. Hill. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, New York.

Giardina, C., R. Sanford, I. Døckersmith, and V. Jaramillo. 2000. The effects of slash burning on ecosystem nutrients during the land preparation phase of shifting cultivation. Plant and Soil **220**:247-260.

Gibson, L., et al. 2011. Primary forests are irreplaceable for sustaining tropical biodiversity. Nature **478**:378-381.

Hill, M. O. 1973. Diversity and Evenness: A Unifying Notation and Its Consequences. Ecology **54**:427-432.

Huse, S. M., D. M. Welch, H. G. Morrison, and M. L. Sogin. 2010. Ironing out the wrinkles in the rare biosphere through improved OTU clustering. Environmental Microbiology **12**:1889-1898.

Islam, K. R. and R. R. Weil. 2000. Land use effects on soil quality in a tropical forest ecosystem of Bangladesh. Agriculture, Ecosystems & Environment **79**:9-16.

Jesus, E. C., T. L. Marsh, J. M. Tiedje, and F. M. S. Moreira. 2009. Changes in land use alter the structure of bacterial communities in Western Amazon soils. The ISME Journal **3**:1004-1011.

Jones, R. T., M. S. Robeson, C. L. Lauber, M. Hamady, R. Knight, and N. Fierer. 2009. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. The ISME Journal **3**:442-453.

Jost, L. 2006. Entropy and diversity. Oikos **113**:363-375.

Jost, L. 2007. Partitioning diversity into independent alpha and beta components. Ecology **88**:2427-2439.

Kaiser, C., M. Koranda, B. Kitzler, L. Fuchslueger, J. Schnecker, P. Schweiger, F. Rasche, S. Zechmeister-Boltenstern, A. Sessitsch, and A. Richter. 2010. Belowground carbon allocation by

trees drives seasonal patterns of extracellular enzyme activities by altering microbial community composition in a beech forest soil. The New Phytologist **187**:843-858.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Applied and Environmental Microbiology **75**:5111-5120.

Lee-Cruz, L., D. P. Edwards, B. M. Tripathi, and J. M. Adams. 2013. Impact of logging and forest conversion to oil palm plantations on soil bacterial communities in Borneo. Applied and Environmental Microbiology **79**:7290-7297.

Legendre, P., Gallagher, E. D. 2001. Ecologically meaningful transformations for ordination of species data. Oecologia **129**:271-280.

Legendre, P., Cáceres, M. 2013. Beta diversity as the variance of community data: dissimilarity coefficients and partitioning. Ecology Letters **16**:951-963.

Lupwayi, N. Z., M. A. Arshad, R. H. Azooz, and Y. K. Soon. 2009. Soil microbial response to wood ash or lime applied to annual crops and perennial grass in an acid soil of northwestern Alberta. Canadian Journal of Soil Science **89**:169-177.

Malhi, Y., T. A. Gardner, G. R. Goldsmith, M. R. Silman, and P. Zelazowski. 2014. Tropical Forests in the Anthropocene. Annual Review of Environment and Resources **39**:125-159.

Marty, F., J. F. Ghiglione, S. Païssé, H. Gueuné, M. C. M. V. Loosdrecht, and G. Muyzer. 2012. Evaluation and optimization of nucleic acid extraction methods for the molecular analysis of bacterial communities associated with corroded carbon steel. Biofouling **28**:363-380.

McGuire, K. L., and K. K. Treseder. 2010. Microbial communities and their relevance for ecosystem models: Decomposition as a case study. Soil Biology and Biochemistry **42**:529-535.

Mendes, L. W., S. M. Tsai, A. A. Navarrete, M. de Hollander, J. A. van Veen, and E. E. Kuramae. 2015. Soil-Borne Microbiome: Linking Diversity to Function. Microbial Ecology.

1 Moreira, F. M. S., R. S. A. Nóbrega, E. D. C. Jesus, D. F. Ferreira, and D. V. Pérez. 2009. Differentiation

2   in the fertility of Inceptisols as related to land use in the upper Solimões river region, western

3   Amazon. Science of the Total Environment **408**:349-355.

4 Naeem, S. 1998. Species redundancy and ecosystem reliability. Conservation Biology **12**:39-45.

5 Naeem, S., and S. Li. 1997. Biodiversity enhances ecosystem reliability. Nature **390**:507-509.

6 Navarrete, A. A., E. E. Kuramae, M. de Hollander, A. S. Pijl, J. A. van Veen, and S. M. Tsai. 2013.

7   Acidobacterial community responses to agricultural management of soybean in Amazon forest

8   soils. FEMS Microbiology Ecology **83**:607-621.

9 Nielsen, M. N., and A. Winding. 2002. Microorganisms as Indicators of Soil Health. National

10   Environmental Research Institute, Aarhus, Denmark.

11 Ohno, T., and M. Susan Erich. 1990. Effect of wood ash application on soil pH and soil test nutrient

12   levels. Agriculture, Ecosystems & Environment **32**:223-239.

13 Pinheiro, J., and D. M. Bates. 2000. Mixed-effects models in S and S-PLUS. Springer, New York, NY.

14 Prober, S. M., et al. 2015. Plant diversity predicts beta but not alpha diversity of soil microbes across

15   grasslands worldwide. Ecology Letters **18**:85-95.

16 Quesada, C. A., J. Lloyd, L. O. Anderson, N. M. Fyllas, M. Schwarz, and C. I. Czimczik. 2011. Soils of

17   Amazonia with particular reference to the RAINFOR sites. Biogeosciences **8**:1415-1440.

18 Quesada, C. A., et al. 2012. Basin-wide variations in Amazon forest structure and function are mediated

19   by both soils and climate. Biogeosciences **9**:2203-2246.

20 R Development Core Team. 2013. R: a language and environment for statistical computing. R Foundation

21   for Statistical Computing, Vienna, Austria.

22 Rodrigues, J. L. M., et al. 2013. Conversion of the Amazon rainforest to agriculture results in biotic

23   homogenization of soil bacterial communities. Proceedings of the National Academy of Sciences

24   of the United States of America **110**:988-993.

Rousk, J., E. Bååth, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal **4**:1340-1351.

Rousk, J., P. C. Brookes, and E. Bååth. 2009. Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. Applied and Environmental Microbiology **75**:1589-1596.

Sanchez, P. A. 1976. Properties and Management of Soils in the Tropics. JohnWiley and Sons, New York, NY.

Sanchez, P. A., J. H. Villachica, and D. E. Bandy. 1983. Soil Fertility Dynamics after Clearing a Tropical Rainforest in Peru1. Soil Science Society of America Journal **47**:1171-1171.

Solar, R. R. C. et al. 2015. How pervasive is biotic homogenization in human-modified tropical forest landscapes? Ecology Letters: **18**:1108-1118.

Solomon, D. et al. 2007. Long-term impacts of anthropogenic perturbations on dynamics and speciation of organic carbon in tropical forest and subtropical grassland ecosystems. Global Change Biology **13**:511-530.

Thomson, B. C., N. Ostle, N. McNamara, M. J. Bailey, A. S. Whiteley, and R. I. Griffiths. 2010. Vegetation affects the relative abundances of dominant soil bacterial taxa and soil respiration rates in an upland grassland soil. Microbial Ecology **59**:335-343.

Thomson, B. C., N. J. Ostle, N. P. McNamara, S. Oakley, A. S. Whiteley, M. J. Bailey, and R. I. Griffiths. 2013. Plant soil interactions alter carbon cycling in an upland grassland soil. Frontiers in Microbiology **4**:253-253.

Tripathi, B. M., M. Kim, D. Singh, L. Lee-Cruz, A. Lai-Hoe, A. N. Ainuddin, R. Go, R. A. Rahim, M. H. A. Husni, J. Chun, and J. M. Adams. 2012. Tropical soil bacterial communities in Malaysia: pH dominates in the equatorial tropics too. Microbial Ecology **64**:474-484.

1   Turner, I. M. 1996. Species loss in fragments of tropical rain forest: a review of the evidence. Journal of

2   Applied Ecology **33**:200-209.

3   Waldrop, M. P., and M. K. Firestone. 2006. Response of microbial community composition and function

4   to soil climate change. Microbial Ecology **52**:716-724.

5   Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naïve Bayesian classifier for rapid

6   assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental

7   Microbiology **73**:5261-5267.

8   Wardle, D. A. 2006. The influence of biotic interactions on soil biodiversity. Ecology Letters **9**:870-886.

9   Wardle, D. A., L. R. Walker, and R. D. Bardgett. 2004. Ecosystem properties and forest decline in

10   contrasting long-term chronosequences. Science **305**:509-513.

11 Whittaker, R. H. 1972. Evolution and Measurement of Species Diversity. Taxon **21**:213-251.

12 Yachi, S., and M. Loreau. 1999. Biodiversity and ecosystem productivity in a fluctuating environment: the

13   insurance hypothesis. Proceedings of the National Academy of Sciences of the United States of

14   America **96**:1463-1463.

15 Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Mixed effects models and

16   extensions in ecology with R. Springer, New York, NY.

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1 FIGURE LEGENDS
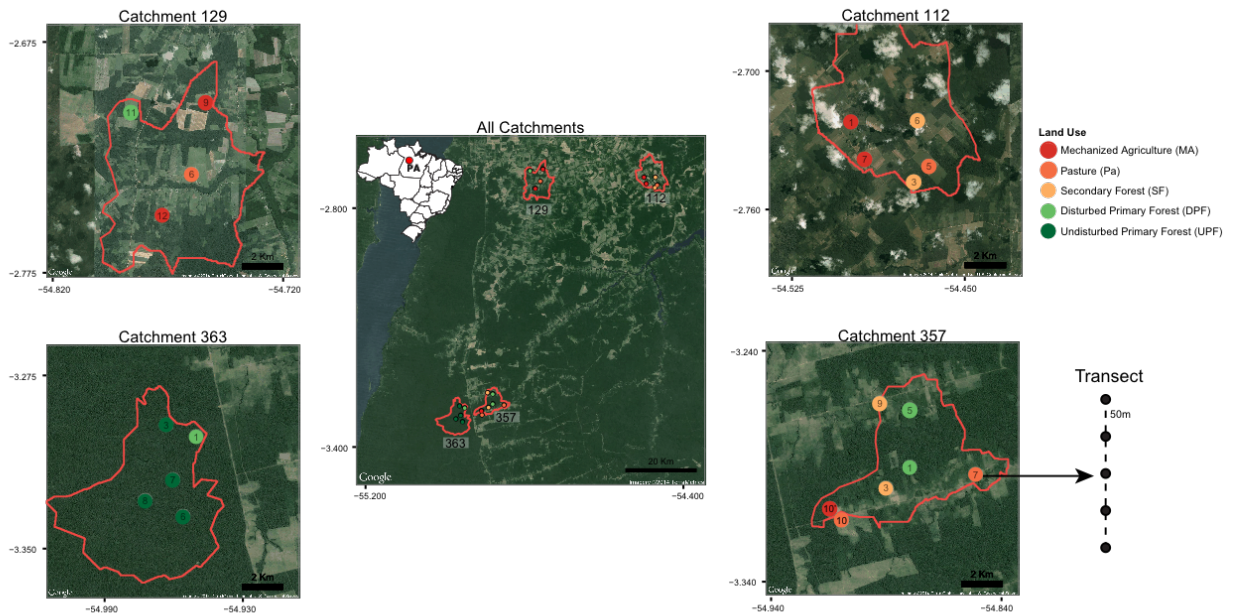
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3 **Figure 1.** Satellite images of the four catchments used in this study. Each point represents one

4 transect with five sampling units spaced by 50 meters. Point colors indicate the land use for each

5 transect as shown in the legend. The x and y axis represent the latitude and longitude, respectively,

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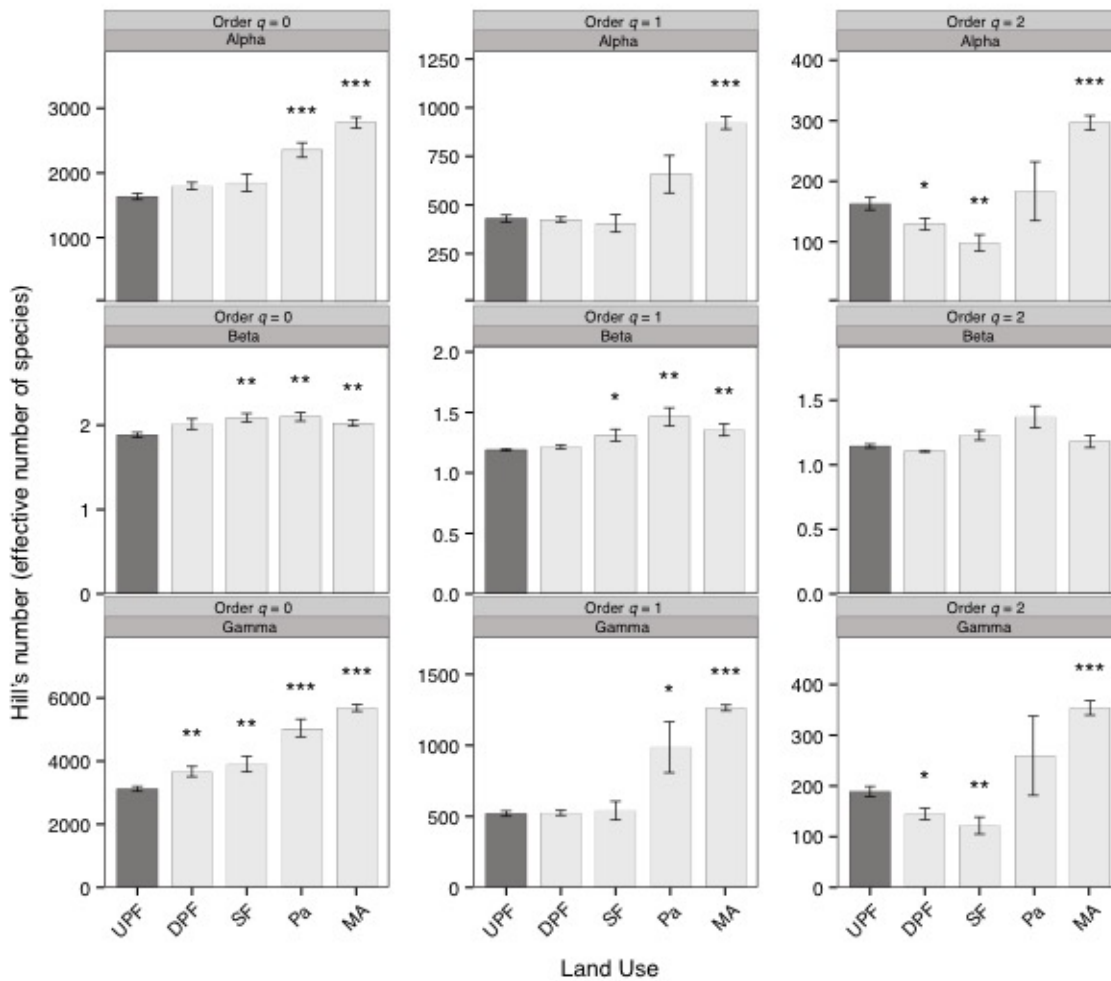
Figure 2. Alpha, beta and gamma bacterial diversity for all OTUs (q = 0), "typical" OTUs (q = 1), and dominant OTUs (q = 2). The bar height indicates the mean for each land use system and th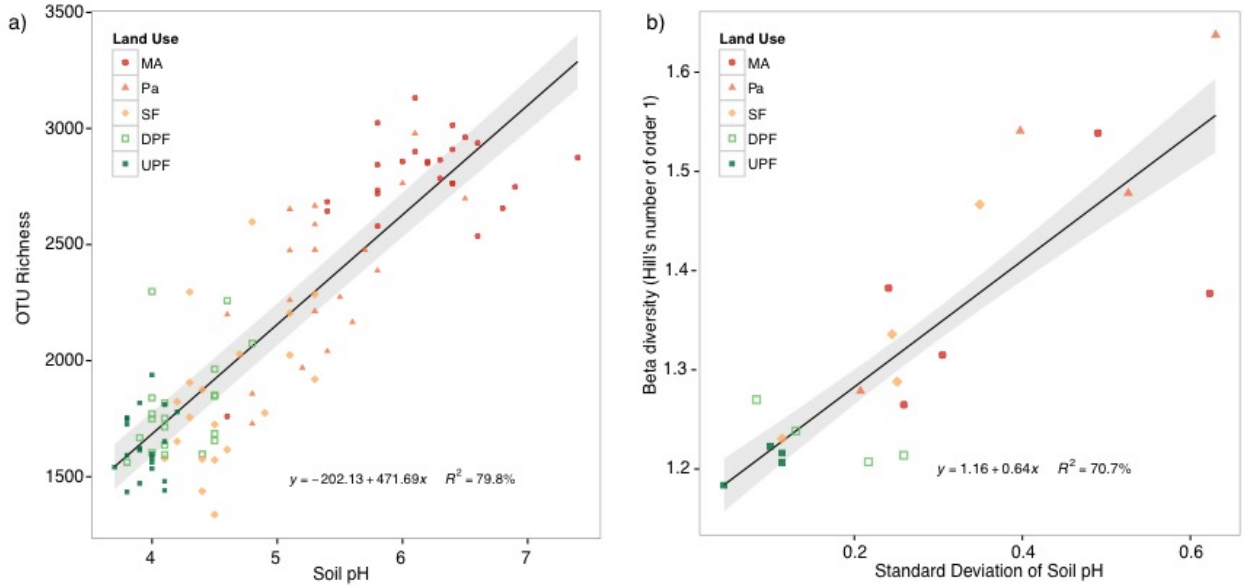e error bar shows the standard error of the mean (UPF, undisturbed primary forest, n = 4; DPF, disturbed primary forest, n = 4; SF, secondary forest, n = 4; Pa, pasture, n = 4; MA, mechanized agriculture, n = 5). The means for each land use is compared to the reference (UPF), in dark grey, using generalized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

1

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.

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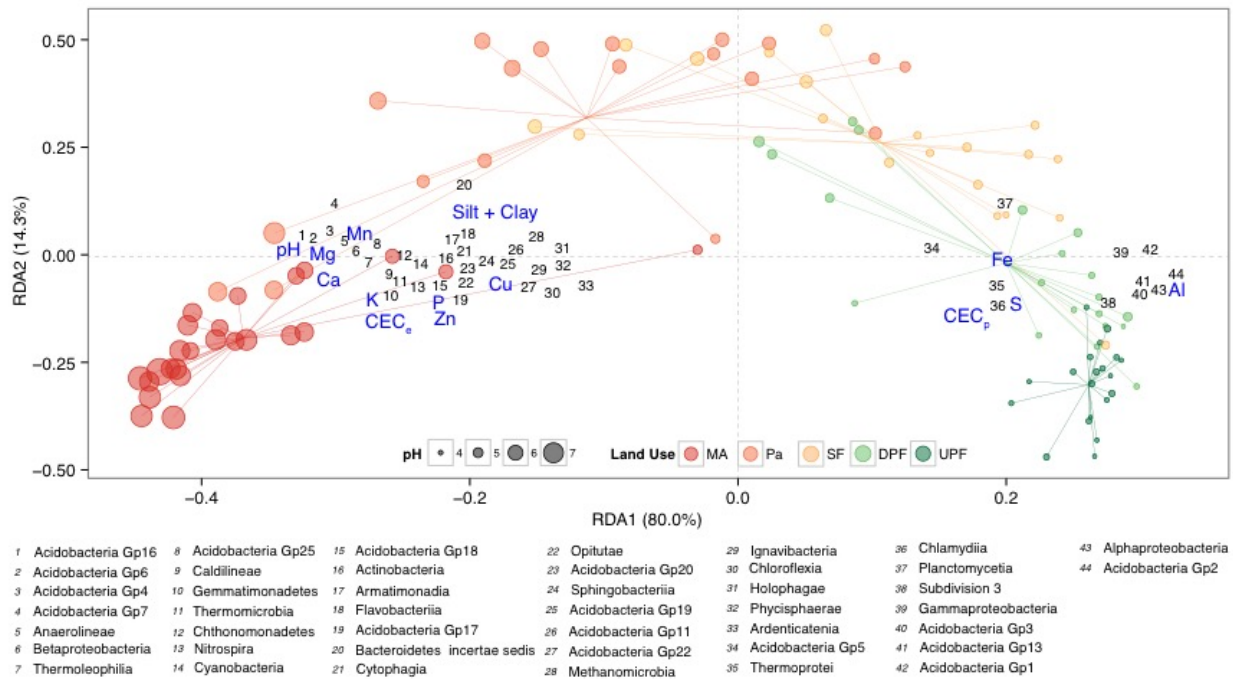
Figure 4. Redundancy analysis of the effect of land use on the abundance of soil bacteria in the eastern Amazon. Taxonomic classes (in black) and soil variables (in blue) were projected on the ordination axis using Kendall rank and Pearson correlation, respectively, and only those with significant correlation ($P \leq 0.05$; 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.

1 **Figure 5**. Heatmap of (center log-ratio) scaled relative abundances of the most generalist OTUs

2 (occurrence > 20% for all sites). Among the 4,893 OTUs that fell into this category, only those

3 significantly affected by land use (Kruskal-Wallis test; $P < 0.05$ after false discovery correction)

4 are shown (3,776 in total). The OTUs are arranged in rows; the samples, in columns; and the cell

5 colors indicate the scaled relative abundance according to the legend at bottom. Rows are grouped

6 into taxonomic classes as indicated in the right axis, and columns are grouped into land uses, as

7 indicated at top. UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary

8 forest; Pa, pasture; MA, mechanized agriculture

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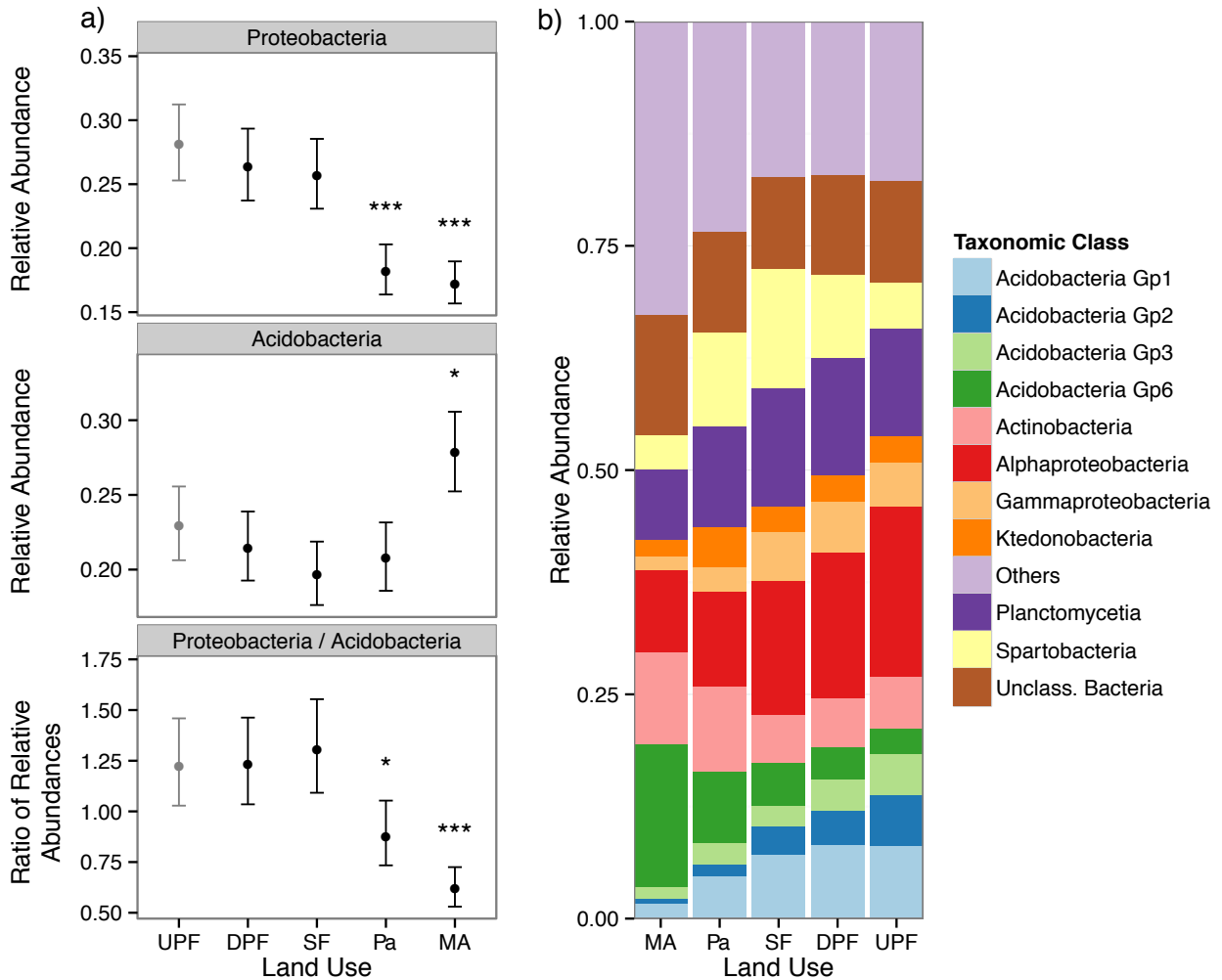
1 **Figure 6**. Dominant bacterial taxa in eastern Amazon soils. a) Mean relative abundances of the

2 phylum *Proteobacteria*, *Acidobacteria*; and Protebacteria:*Acidobacteria* ratio. Vertical bars

3 indicate the 95% confidence interval calculated using MCMC (10,000 simulations). Means are

4 compared to UPF (in grey). * Significant at $P \le 0.05$; *** Significant at $P \le 0.001$. b) Relative

5 abundances of the dominant taxonomic classes. UPF, undisturbed primary forest; DPF, disturbed

6 primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture.
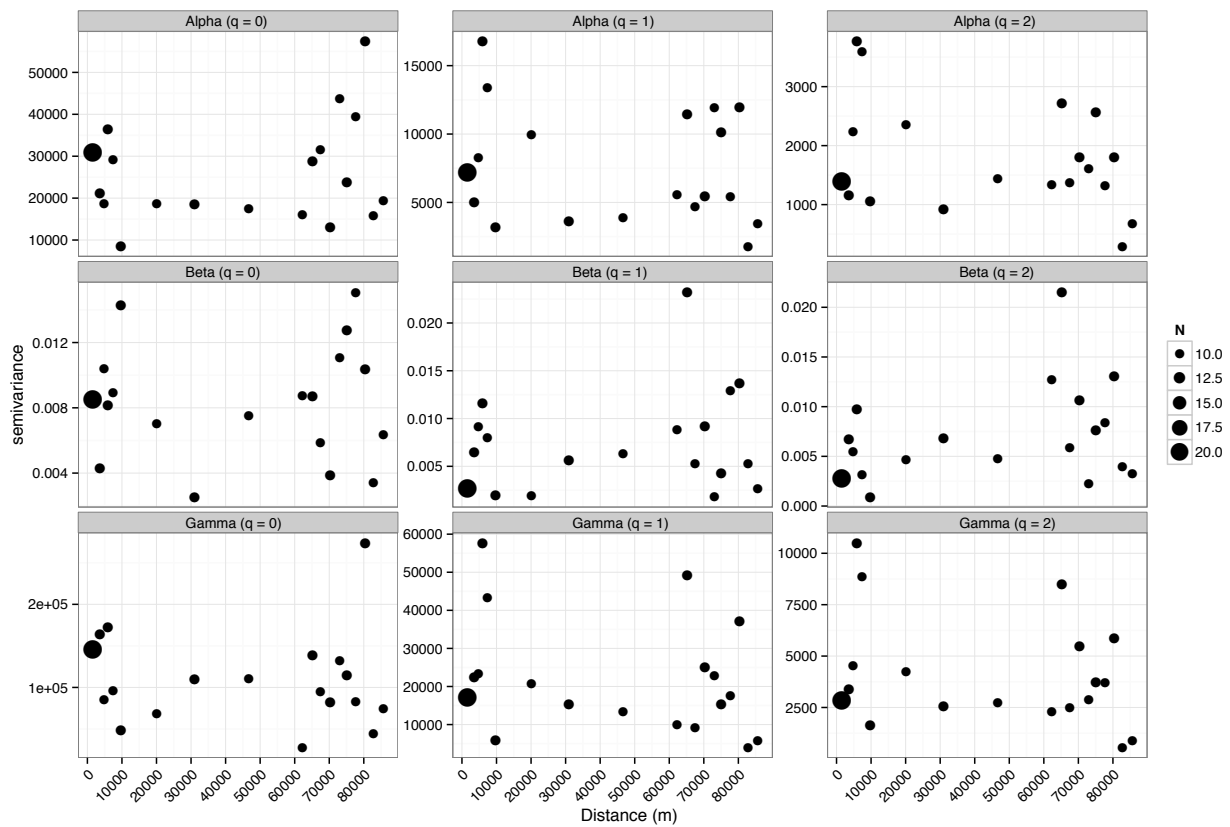
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Teotonio S. de Carvalho, Ederson C. Jesus, Jos Barlow, Toby A. Gardner, Isaac C. Soares, James M. Tiedje, Fatima M.S. Moreira. 2015. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. *Ecology*.

APPENDIX S1. Semivariograms for alpha, beta, and gamma diversity.

Figure S1. Semivariograms for alpha, beta and gamma bacterial diversity for all OTUs (q = 0), "typical" OTUs (q = 1), and dominant OTUs (q = 2). The point size indicates the number of pairwise distances used to calculate each semivariance according to the legend at right.

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Teotonio S. de Carvalho, Ederson C. Jesus, Jos Barlow, Toby A. Gardner, Isaac C. Soares, James M. Tiedje, Fatima M.S. Moreira. 2015. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. *Ecology*.
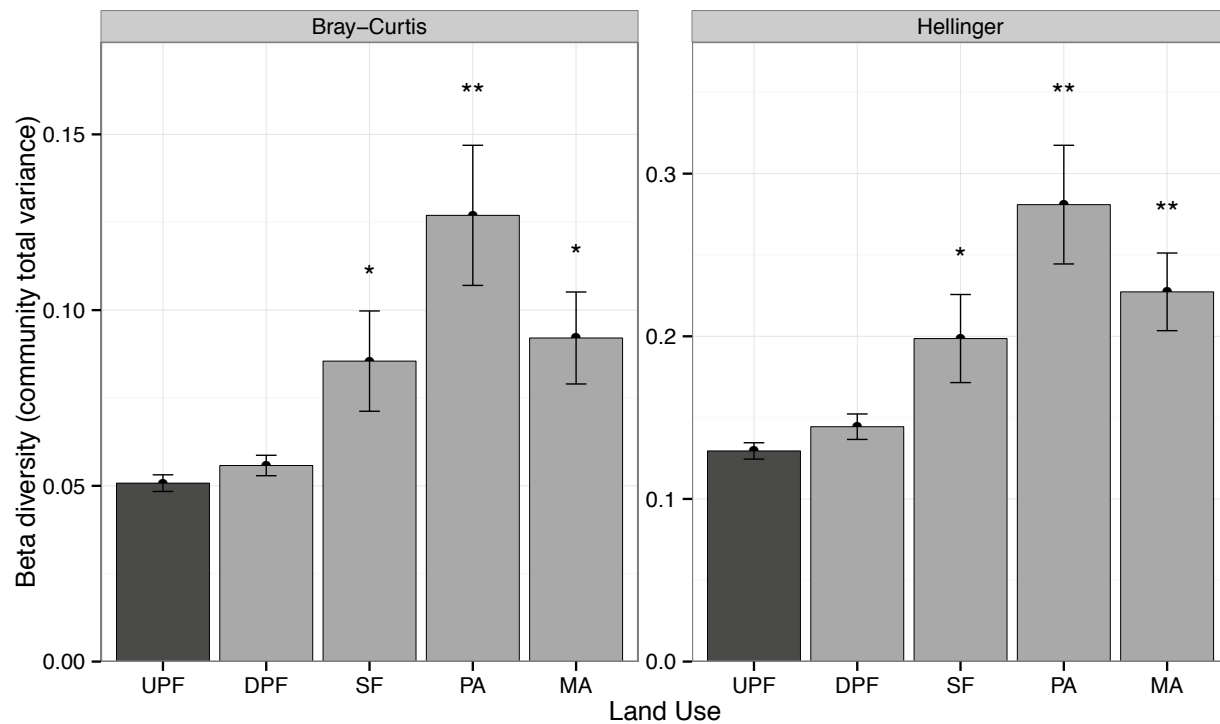
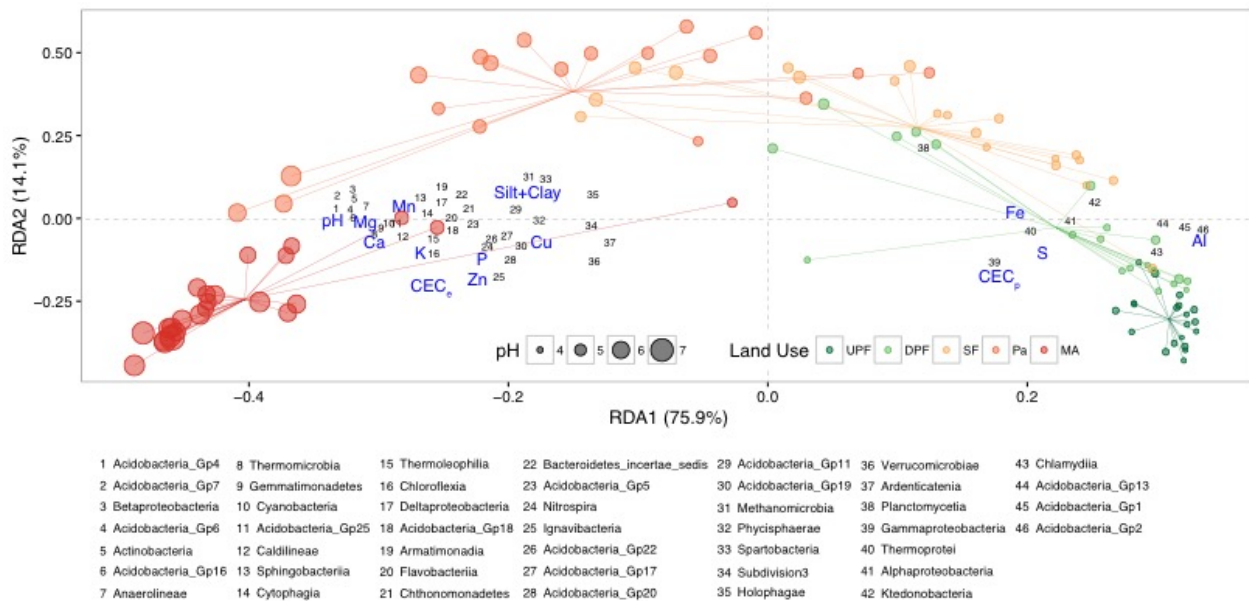APPENDIX S2. Beta diversity calculated as total community variance

Figure S1. Beta diversity (total community variance) of soil bacterial communities in the eastern Amazon. The bar height indicates the mean of beta diversity based on Bray-Curtis dissimilarity (left panel) and Hellinger distance (right panel) 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 were compared to the reference (UPF), in dark grey, using generalized least squares. * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$.

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Teotonio S. de Carvalho, Ederson C. Jesus, Jos Barlow, Toby A. Gardner, Isaac C. Soares, James M. Tiedje, Fatima M.S. Moreira. 2015. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. *Ecology*.

APPENDIX S4. Redundancy analysis based on Hellinger-transformed presence-absence data

1 Acidobacteria_Gp4	8 Thermomicrobia	15 Thermoleophilia	22 Bacteroidetes_incertae_sedis	29 Acidobacteria_Gp11	36 Verrucomicrobiae	43 Chlamydiia
2 Acidobacteria_Gp7	9 Gemmatimonadetes	16 Chloroflexia	23 Acidobacteria_Gp5	30 Acidobacteria_Gp19	37 Ardenticatenia	44 Acidobacteria_Gp13
3 Betaproteobacteria	10 Cyanobacteria	17 Deltaproteobacteria	24 Nitrospira	31 Methanomicrobia	38 Planctomycetia	45 Acidobacteria_Gp1
4 Acidobacteria_Gp6	11 Acidobacteria_Gp25	18 Acidobacteria_Gp18	25 Ignavibacteria	32 Phycisphaerae	39 Gammaproteobacteria	46 Acidobacteria_Gp2
5 Actinobacteria	12 Caldilineae	19 Armatimonadia	26 Acidobacteria_Gp22	33 Spartobacteria	40 Thermoprotei	
6 Acidobacteria_Gp16	13 Sphingobacteriia	20 Flavobacteriia	27 Acidobacteria_Gp17	34 Subdivision3	41 Alphaproteobacteria	
7 Anaerolineae	14 Cytophagia	21 Chthonomonadetes	28 Acidobacteria_Gp20	35 Holophagae	42 Ktedonobacteria	

Figure S1. Redundancy analysis of the effect of land use on the occurrence (Hellinger-transformed presence-absence data) 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 (21% of the total variation) that is represented by the respective axis. CEC_p, potential cation exchange capacity; CEC_e, effective cation exchange capacity.

Teotonio S. de Carvalho, Ederson C. Jesus, Jos Barlow, Toby A. Gardner, Isaac C. Soares, James M. Tiedje, Fatima M.S. Moreira. 2015. Land use intensification in the humid tropics increased both alpha and beta diversity of soil bacteria. *Ecology*.

APPENDIX S5. Phylogenetic and compositional dissimilarities among soil bacterial communities across a gradient of land use intensity in the eastern Amazon.

Table S1.

Pair-wise contrasts assessing phylogenetic and compositional dissimilarities among soil bacterial communities along a gradient of land use intensity in the eastern Amazon.

Contrast[1]	Phylogenetic Dissimilarity (Unifrac)[2]		Compositional Dissimilarity (Hellinger)[3]	
	Unweighted	Weighted	Presence/ Absence	Relative Abundance
Pa *vs* MA	1.5	2.3	4.5*	9.4*
DPF *vs* MA	63.3**	91.0**	14.0**	34.9**
DPF *vs* Pa	1.2*	0.9^{ns}	5.2*	9.7*
UPF *vs* MA	110.3**	123.8*	19.1**	48.0**
UPF *vs* PA	0.5^{ns}	0.6^{ns}	8.6*	17.4*
UPF *vs* DPF	5.3*	5.1*	2.0*	3.9*
SF *vs* MA	40.5**	64.3**	10.9**	25.9**
SF *vs* Pa	0.7^{ns}	0.5^{ns}	3.5*	6.0*
SF *vs* DPF	3.2*	2.8*	1.9*	3.1*
SF *vs* UPF	13.0*	9.9*	4.4*	10.1*

[1]UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; Pa, pasture; MA, mechanized agriculture.

[2]*F*-statistic calculated by PERMANOVA on the (unweighted or weighted) Unifrac distance matrix. ns, non-significant; *, $P < 0.05$; **, $P < 0.01$. The significance of each contrast was calculated after 1,000 permutations. Transects were permuted as a whole to account for the nesting of sampling points within transects (see Figure 1).

[3]*F*-statistic calculated by RDA after Hellinger transformation on the presence-absence or relative abundance data. The significance of each contrast was calculated as above.
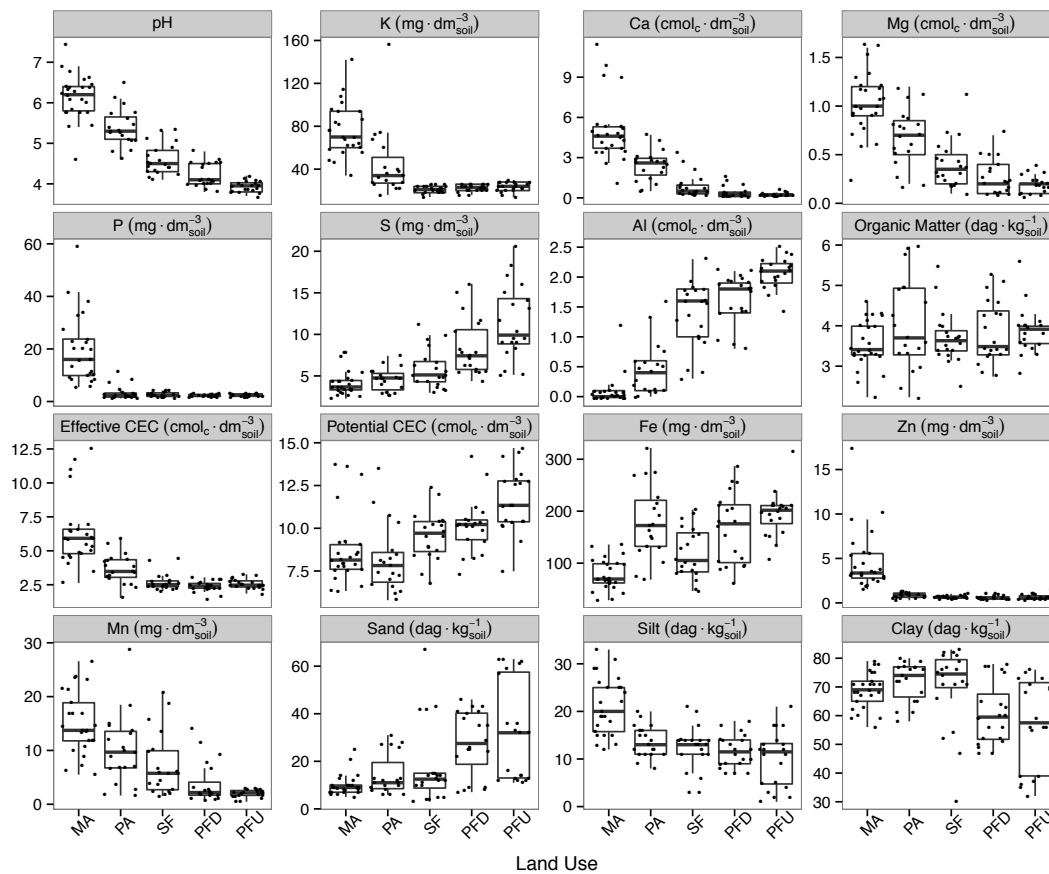
Teotonio S. de Carvalho, Ederson C. Jesus, Jos Barlow, Toby A. Gardner, Isaac C. Soares, James

M. Tiedje, Fatima M.S. Moreira. 2015. Land use intensification in the humid tropics increased

both alpha and beta diversity of soil bacteria. *Ecology*.

APPENDIX S6. Metagenome predictions using PICRUSt

1

APPENDIX S3. Soil properties in the sampled sites.

Figure S1. Boxplots of physicochemical parameters of soils from eastern Amazon. Points were jittered to facilitate visualization. UPF, undisturbed primary forest; DPF, disturbed primary forest; SF, secondary forest; PA, pasture; MA, mechanized agriculture.

2