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TESE

Alterações Edáficas, Microbianas e na Saúde Animal Decorrentes da Conversão Floresta-Pastagem na Amazônia Ocidental

Fernando Igne Rocha

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UNIVERSIDADE FEDERAL RURAL DO RIO DE JANEIRO
INSTITUTO DE AGRONOMIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA
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ALTERAÇÕES EDÁFICAS, MICROBIANAS E NA SAÚDE ANIMAL DECORRENTES DA CONVERSÃO FLORESTA-PASTAGEM NA AMAZÔNIA OCIDENTAL

FERNANDO IGNE ROCHA

Sob a Orientação do Pesquisador
Ederson da Conceição Jesus

e Coorientação do Pesquisador
Wenceslau Geraldes Teixeira

Tese submetida como requisito parcial para obtenção do grau de **Doutor** no Programa de Pós-Graduação em Agronomia – Ciência do Solo, Área de Concentração em Biologia do Solo.

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TESE APROVADA EM 17/12/2021.

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"*… And it's whispered that soon*
If we all call the tune
Then the piper will lead us to reason
And a new day will dawn
For those who stand long
And the forests will echo with laughter…"

Robert Plant

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BIOGRAFIA

Fernando Igne Rocha, nascido em 09 de agosto de 1990, na cidade de Cuiabá, Mato Grosso, Brasil. Filho de Ivani Igne e Fernando Luiz Franco Rocha, e irmão de Camila Igne Rocha, Raquel Igne Rocha, Alexandre Igne Rocha, e Victor Igne Franco Rocha. Concluiu o ensino fundamental no ano de 2005, na Escola Municipal Frederico Trotta, RJ, ingressando para o ensino médio-técnico profissionalizante em Gestão Administrativa, na Escola Técnica Estadual Adolpho Bloch – FAETEC, RJ. Em março de 2009 iniciou seus estudos em Agronomia, na Universidade Federal Rural do Rio de Janeiro. No mesmo semestre integrou o laboratório de Biologia do Solo/ Depto de Solos como estagiário voluntário, sob orientação do prof. Dr. Ricardo L. L. Berbara. Entre 2010 e 2015, realizou atividades de pesquisa na Embrapa Agrobiologia, como bolsista de IC do laboratório de Ecologia de Paisagens Agrícola, sob orientação da Dra. Mariella C. Uzêda. Entre 2012-2013 realizou graduação-sanduíche na Universidad de Vigo, Galícia, Espanha, pelo até então extinto programa Ciência sem Fronteiras (processo 207825/2012-2), realizando um período de estágio no laboratório de Biologia Vegetal e Ciência do Solo da mesma Universidade, sob orientação da Dra. Nuria Pedrol Bonjoch. Entre 08/2015 - 07/2017 realizou o mestrado pelo Programa de Pós-Graduação em Fitotecnia/ UFRRJ, bolsista CAPES, sob orientação do Dr. Aroldo F. L. Machado e Dra. Mariella C. Uzêda. Em setembro de 2017 iniciou suas atividades como doutorando do Programa de Pós-Graduação em Agronomia – Ciência do Solo/ UFRRJ, bolsista CNPq (processo 165571/2017-9), sob orientação do Dr. Ederson da Conceição Jesus e co-orientação do Dr. Wenceslau Geraldes Teixeira. Atuou como representante discente entre 11/2018 - 02/2021. Entre 11/2019 - 11/2020 realizou o período de doutorado-sanduíche na Iowa State University, Estados Unidos, com bolsa de estudos pelo Programa de Doutorado-Sanduíche no Exterior - CAPES (processo 88881.361652/2019-01), sendo supervisionado pela Dra. Adina Howe, do Genomics and Environmental Research in Microbial Systems lab. Em dezembro de 2021 submete a presente tese de doutorado para a apreciação dos membros de sua banca examinadora.

RESUMO GERAL

ROCHA, Fernando Igne. **Alterações edáficas, microbianas e na saúde animal decorrentes da conversão floresta-pastagem na Amazônia Ocidental**. 2021. 156f. Tese (Doutorado em Agronomia - Ciência do Solo). Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2021.

A pedosfera, camada terrestre de extremo dinamismo entre fatores bióticos e abióticos, expressa as digitais da relação evolutiva humano x natureza e tem no século XX a intensificação de seu uso, culminando no alerta global sobre os *hotspots* de biodiversidade. Não diferentemente, a Amazônia está sendo largamente submetida a tal processo, tendo o seu ponto de inflexão - *tipping point* - já sido alertado. O advento do sequenciamento de próxima geração vêm marcando significativo avanço no reconhecimento de microorganismos não-detectáveis por ferramentas de microbiologia clássica. O uso de tais métodos possibilitou compreender como a conversão floresta-pastagem afeta o funcionamento de microbiomas edáficos. Contudo, ainda pouco se sabe qual o efeito-cascata de tais mudanças. É pioneirismo do Dr. Jürgen Döbereiner reportar que surtos de periodontite em bovinos na Amazônia (i.e., cara-inchada - CIb) estariam associados às transformações na microbiota de solos pela abertura ou renovação de pastagens. Contudo, os métodos disponíveis à época limitaram explorar tal hipótese. Aqui, regiões na Amazônia Ocidental brasileira, com alta importância para a conservação da biodiversidade foram acessadas. Florestas e pastagens adjacentes, em fazendas caracterizadas como de baixo e alto nível de severidade (BNS e ANS) da periodontite bovina, foram selecionadas. Os solos foram caracterizados, e por meio do sequenciamento do gene 16S rRNA, explorou-se a microbiota associada ao chão da floresta (i.e., serrapilheira, camada de raíz, e solo mineral), bem como ao solo de pastagens, a forragem, e ao biofilme subgengival de bovinos sadios e doentes. Em geral, a magnitude de transformação das variáveis do solo foi maior naqueles mais intemperizados, no entanto a soma de bases foi consistentemente maior em todas as pastagens. Corroboramos que, independente do tipo de solo, a diversidade alfa microbiana é positivamente correlacionada com o pH do solo. Contudo, a avaliação conjunta das camadas do chão da floresta evidenciou que este abriga uma maior heterogeneidade espacial (diversidade beta) do que solos de pastagem. O estudo do continuum solo-planta-animal ressaltou que pastagens BNS possuem predominância das classes *Bacilli* e *Gammaproteobacteria*, maiores teores de Cu nos solos, e de macro e microminerais na forragem. Pastagens ANS apresentaram maior abundância de *Bacteroidia* na forragem e animais, e *Actinobacteria* nos solos, e maior relação C:N. Ademais, o continuum do sistema ANS apresentou maior abundância relativa do gene de biossíntese de estreptomicina, relatado por facilitar a aderência do patógeno às células epiteliais. A maior diversidade alfa e gama, e de modularidade na análise de redes, indicaram que a microbiota de pastagens ANS está em maior distúrbio (disbiose). Por fim, o estudo de fluxos hídricos para a região de Boca do Acre/AM apontou que solos das pastagens do sistema ANS mantém graus de saturação próximos a 90% na estação chuvosa, expondo a forragem e o gado a maiores eventos de umidade, com possível redução de O_2 para o metabolismo vegetal e microbiano. A abordagem multidisciplinar aqui utilizada possibilitou avançar na compreensão da complexidade associada ao efeito-cascata da conversão floresta-pastagem e impactos sobre a biodiversidade microbiana e saúde animal.

Palavras-chave: Amazônia. Pedodiversidade. Biodiversidade do solo. Mudança do uso da terra. Saúde do gado.

GENERAL ABSTRACT

ROCHA, Fernando Igne. **Edaphic, microbial, and animal health changes due to forest-to-pasture conversion in Western Amazonia**. 2021. 156p. Thesis (Doctor of Science in Agronomy, Soil Science). Instituto de Agronomia, Universidade Federal Rural do Rio de Janeiro, Seropédica, RJ, 2021.

The pedosphere, a terrestrial layer of extreme dynamism between biotic and abiotic factors, expresses the fingerprints of the evolutionary relationship between humans *x* nature, and in the 20th century has seen an accelerated intensification of its use, culminating in the global alert about biodiversity hotspots. Not differently, the Amazonia is being largely submitted to such process, and the proximity to its tipping point has already been alerted. The advent of next-generation sequencing has marked a significant advance in the recognition of microorganisms undetectable by classical microbiology tools. The use of such methods has made it possible to understand how forest-to-pasture conversion affects the composition, diversity, and function of edaphic microbiomes. However, little is yet known about the cascading-effect driven by such changes. The pioneering work of Dr. Jürgen Döbereiner reported that outbreaks of periodontitis in cattle (i.e., cara-inchada - CIb) are associated with transformations in the soil microbiota by opening or renewing pastures for extensive cattle grazing. However, available molecular methods have not made it possible to explore these observations more deeply. Here, regions with high importance for biodiversity conservation and immersed in a gradient of land-use change in Brazilian Western Amazonia were accessed. Forests and adjacent pastures on farms characterized as low and high severity level (LSL and HSL) of cattle periodontitis were selected. We characterized the soils, and through 16S rRNA gene sequencing, we explored the associated forest floor microbiota (i.e., litter, root layer, and bulk soil), as well as pasture soil, forage, and subgingival biofilm from healthy and diseased cattle. Overall, the magnitude of transformation of soil variables is greater in those with high weathering degree, however, the sum of bases was consistently higher in pastures. We corroborate that, regardless of soil class, the microbial alpha diversity follows positive correlation with soil pH, moreover, the joint evaluation of the forest floor layers evidenced that it harbors greater spatial heterogeneity (beta diversity) than the microbiota of pasture soils. The soil-plant-animal continuum study highlighted that LSL systems have a predominance of *Bacilli* and *Gammaproteobacteria* classes, higher copper contents in soils, and macro and micromineral contents in forage. HSL systems had higher abundance of *Bacteroidia* in forage and animals, and *Actinobacteria* in soils, and higher C:N ratio. Furthermore, the ANS system continuum showed higher relative abundance of the streptomycin biosynthesis gene, reported to facilitate adhesion of pathogenic bacteria to epithelial cells. The higher alpha and gamma diversity, and modularity in network analysis, indicate that the microbiota of HSL system is reflecting greater environmental stress, characterizing their dysbiosis. Finally, the study of water fluxes for the Boca do Acre/AM region pointed out that soils of pastures in the HSL system maintain degrees of saturation close to 90% in the rainy season, exposing forage and cattle to greater humidity events, with possible reduction of O_2 for plant and microbial metabolism. The multidisciplinary approach used in this study allowed us to advance our understanding of the complexity associated with the cascade-effect of forest-to-pasture conversion and impacts on microbial biodiversity and animal health.

Keywords: Amazonia. Pedodiversity. Soil biodiversity. Land-use change. Cattle health.

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SUMÁRIO

1. GENERAL INTRODUCTION

The change and intensity of land-use by human intervention, encouraged - among others - by the advance of frontiers to extensive agriculture, are considered the most important drivers for the biodiversity loss in the Brazilian Amazonia (BROWN; BROWN; BROWN, 2016), suggesting that the tipping point is closer than expected (AMIGO, 2020). This issue is widely exposed in scientific studies that correlate the effects of landscape simplification on fauna and plant species (GIBSON et al., 2011; WEARN; REUMAN; EWERS, 2012), however, research considering the edaphic microbiota still receive less attention. Edaphic microbiota is essential for key processes in all ecosystems, and responds in different ways to land-use changes, through alterations in its structure, diversity, composition, and function (NAVARRETE et al., 2015; PAULA et al., 2014; RITTER et al., 2020; TAKETANI; TSAI, 2010). Furthermore, the soil microbial community may be directly and indirectly related to disease dynamics, in response to changes in abundance, demography, immune response, as well as the composition of microbial communities (GOTTDENKER et al., 2014).

Considering the above, the disease commonly reported as "swollen face" or “cara-inchada dos bovinos – CIb” in Portuguese is characterized as a periodontitis associated with strict anaerobic gram-negative microorganisms that affects ruminants (e.g., bovine epizootic periodontitis) and is related to pastures established in newly deforested areas (DÖBEREINER et al., 2000). In addition, the same review indicates that in some regions the disease became active after pasture reform management (i.e.: plowing, fertilization, and seeding), which may be related to the microbial response to direct changes in soil chemical variables. The periodontal disease has been frequently reported in Brazil between the 1960s and 1980s, due to its great importance in the economy and animal health, however, new cases were recently observed in sheep herds in the Amazonia, presenting the same epidemiological conditions and characteristics observed in cattle.

Land-use change significantly favors changes in the structure and composition of soil microbial communities and consequently the dominance of specific populations, such as *Actinobacteria* (DE CARVALHO et al., 2016). Production of sub-inhibitory quantities of streptomycin by *Actinobacteria* has been suggested as one of the possible triggers of the disease, since this leads to an increased adherence of *Bacteroides spp.* to the gingival epithelium and to the progressive destruction of the periodontal tissue (DÖBEREINER; DUTRA; ROSA, 2004). Thus, as forest-to-pasture conversion changes the natural characteristics of the soil (e.g., moisture, texture, TOC, and pH) that drastically drive the structuring of the microbiota, it is necessary to investigate these changes in detail in order to identify possible drivers of periodontitis in ruminants taking advantage of a multidisciplinary research through an ecosystemic perspective.

The general objective of this thesis was to measure the transformations that the soil environment undergoes as a result of forest-to-pasture conversion, and to associate these transformations with the prokaryotic metacommunity. Based on these data, the thesis was developed along two central axes, namely: 1) Effect of land-use change on the biodiversity of the edaphic environment; where we aimed to understand the general changes in the composition of microbial communities after removal of forest cover and consequent loss of habitat (litter, root layer, physicochemical transformations of the bulk soil), as well as the impact of this phenomenon on the scales of microbial diversity (local and regional) by diversity partitioning methods; and 2) Relationship between land-use change, local abiotic factors, and the increased susceptibility of

pastures to trigger periodontitis in cattle. To address this goal, molecular biology tools such as next-generation sequencing of 16S ribosomal RNA gene, as well as real-time quantitative PCR (RT-qPCR), were used to characterize the prokaryotic community and abundance of antibiotic genes in soil, forage, and bovine oral microbiota components, across a different study conditions and regions of incidence of the disease (Figure 1). The use of cultivation-independent methods to study the disease in question is unprecedented. Cattle periodontitis has been extensively studied by Dr. Jürgen Döbereiner (*in memoriam*) for over 50 years, however, mostly by analytical tools based on cultivation-dependent methods, which allow the assessment of a limited ~1% of soil microbial diversity (RUPPERT; KLINE; RAHMAN, 2019).

Thus, the general hypothesis of this thesis is that soil chemical variables in pastures established in recently deforested areas, as well as the management practices of pasture renewal, trigger conditions for antibiotic-producing bacteria to be stimulated, which may cause imbalances in the oral microbiota of ruminants through ingestion of contaminated forage. The answers to the raised hypotheses will be important to contribute to management practices capable of reducing periodontitis outbreaks in ruminants. Moreover, these answers will provide new information with the potential to boost actions aimed at biodiversity conservation in priority regions.

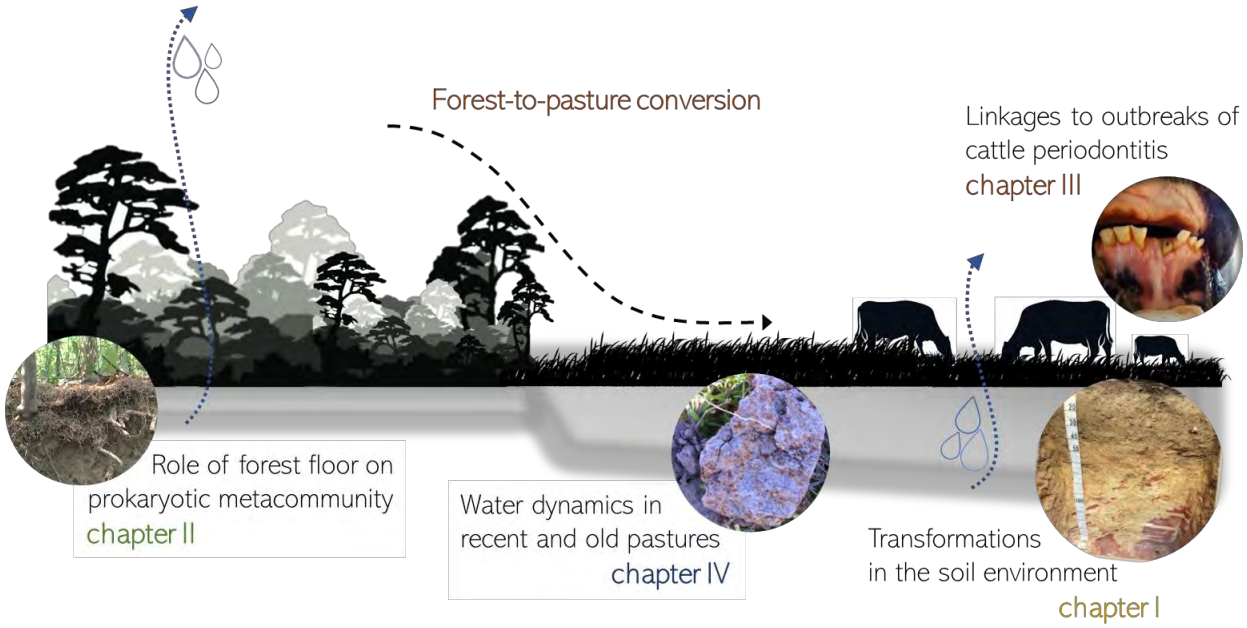

Figure 1. Schematic illustration of the topics addressed in this thesis. Through different perspectives and methods, it was sought to highlight the effects of the forest-to-pasture conversion on the soil environment, microbial biodiversity, soil water flows, as a trigger of processes that, depending on the arrangement between biotic and abiotic drivers, may culminate in the bovine periodontitis disease, historically reported to be rooted in the disruption of soil ecological stability in response to the removal of the original vegetation cover.

2. LITERATURE REVIEW

2.1 Amazonian Soils, and Effects of Anthropogenic Land-Use Change on Soil Properties

Tropical soils can arise from a wide variety of parent materials, climatic conditions, biotic interactions, geomorphology, and age (QUESADA et al., 2011; SOMBROEK, 1966). Particularly, the Andean uplift generated tectonic load and sediment flux into Amazonian lowland, deeply transforming a previously craton dominated land into the diverse edaphic mosaic found in the present period (HOORN et al., 2010).

According to Schaefer et al. (2017), the Amazonia has been divided into 11 pedological mega-sectors, which represent large pedo-environments. The sedimentary basins follow a strong geological-structural control, coinciding with the subdivision of the basins, which determine a huge pedodiversity. Acrisols and Ferralsols in the crystalline areas of the Amazonian craton, are the widespread and more predominant soils thorough the basin, generally dystrophic, except for places with occurrence of mafic rocks (ferromagnesian silicates) (BRAVARD; RIGHI, 1990). Distinctly, the Solimões Formation basically originates four types of predominant soils: Luvisols, Cambisols, Ferralsols, and Acrisols, which makes the state of Acre the most extensive and continuous patch of Luvisols in the Amazonian basin. Luvisols are moderately deep soils, eutrophics, and may be associated with Cambisols and Gleysols at the bottom of the valleys (SCHAEFER et al., 2017). The levels of exchangeable calcium in the soils of Acre are strongly correlated with the sum of bases, which indicates the relevance of this nutrient for the availability of exchangeable bases (BERNINI et al., 2013).

Nevertheless, Amazon rainforest is one of the most oligotrophic of the world, and therefore provides mechanisms for retention and greatly improved nutrient cycling (BRAZ; FERNANDES; ALLEONI, 2013; HERRERA et al., 1978). The oligotrophic characteristic of the forest is especially caused by the high degree of weathering process followed by soil leaching (APRILE et al., 2013).

The forest-to-pasture conversion is an anthropic activity that consists in opening pristine areas to extract valuable wood and then burning the remaining plant cover to introduce annual or perennial crops or to form pasture (ANDREUX; CERRI, 1989). The impacts of deforestation include loss of biodiversity, reduced water cycling (and rainfall), and contributions to changes at global scale (DAVIDSON et al., 2012; FEARNSIDE, 2005). The conversion of primary rainforest to agricultural land or pasture leads to a significant increase of pH and to the reduction of exchangeable aluminum concentrations due to the deposition of base cations through the ashes of combusted forest biomass, as pointed out by many authors (ALFAIA et al., 2004; MOREIRA et al., 2009). However, it is generally time dependent (FARELLA et al., 2007), and closely related to the soil type (MOREIRA; FAGERIA; GARCIA Y GARCIA, 2011; MOREIRA et al., 2009; NUMATA et al., 2007).

McGrath et al. (2001) in a meta-analysis including Ferralsols and Acrisol, also observed that the soil pH and exchangeable calcium were consistently higher in pastures than primary or secondary forest. Moreover, this change in plant cover also is reported to reduce the activity of microbial groups and functions, as well as the microbial biomass, which is one of the main factors for introducing nutrients into the system and energy flow to the soil (ANDREUX; CERRI, 1989; KASCHUK; ALBERTON; HUNGRIA, 2011).

2.2 Landscape Simplification, Effects and Modifications in Edaphic Microbiomes

Forest-to-pasture conversion is considered the main cause of deforestation in the Brazilian Amazonia, mostly in the recent years (Figure 2; ASSIS et al., 2019) due to the lack of environmental policies of the current government for the conservation of the biome, with an increase from 5.3 - 41.81 million cattle herds (680%) and 16.4-52.7 million hectares (221%) converted to pasture between 1985 and 2020 (FRANÇA et al., 2021).

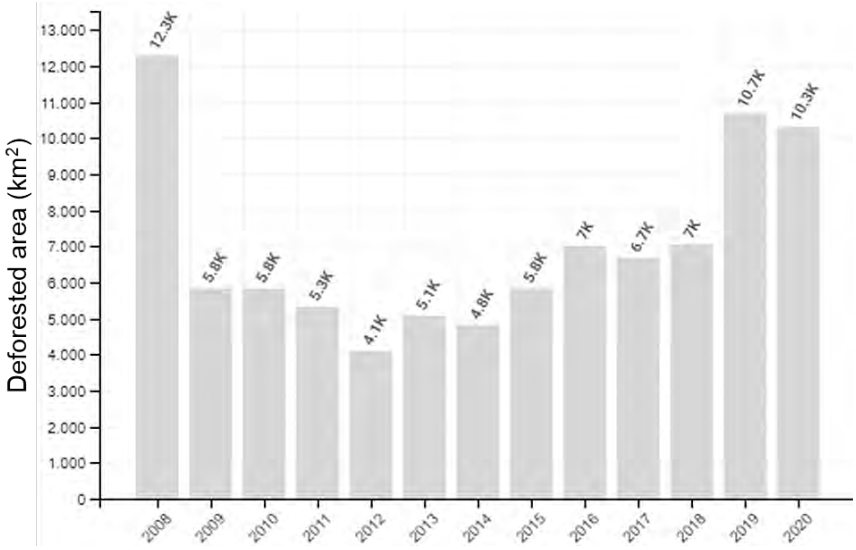

Figure 2. Recent deforestation increment rate in the Brazilian Amazonia (Terra Brasilis, INPE, 2021).

One of the main consequences of this conversion is the impact on soil biodiversity, which was defined as "a measure of environmental quality" and "key to understanding the health of agroecosystems" (FAO, 2014). The transformation of the forest into pasture causes a significant reduction in the absorption of solar radiation and can also affect the climate and the hydrological regime, changing precipitation rates, both at a local and regional scale (DAVIDSON et al., 2012). The use of fire on the vegetation remaining after the conversion of land-use, as a cleaning tool, alters the maintenance of N in the soil, of the C stock, increasing aerosol particles and concentrations of greenhouse gases in the atmosphere (DAVIDSON et al., 2012).

Reducing the scale of observation, the intensification of land-use homogenizes plant communities (ARROYO-RODRÍGUEZ et al., 2013). Consequently, the diversity of substances in the rhizosphere and litter, as well as the diversity of soil microhabitats, and the diversity of plant hosts for symbiotic microorganisms can also be reduced due to this process (WARDLE, 2006). Recent studies have emphasized the threat of forest conversion to pasture for the loss of soil biodiversity (RODRIGUES et al., 2013), however, studies are needed to further understand the real consequences of this loss. In addition, the practice of slash-and-burn and the application of fertilizers and soil amendments considerably alter these already mentioned factors (FIERER; JACKSON, 2006; JESUS et al., 2009). These practices, in turn, have already been identified as important factors influencing soil microbial communities (KURAMAE et al., 2012), directly affecting their ecosystem structure and functions (BARDGETT; VAN DER PUTTEN, 2014). Understanding how the microbial community responds to environmental changes can be useful

from an agronomic point of view, as nutrient cycling is a key process in maintaining agricultural productivity, especially in tropical areas of the globe.

Different types of soil microorganisms seem to respond differently to land-use changes, for example, arbuscular mycorrhizal fungi (STÜRMER; SIQUEIRA, 2011) and soil bacteria (JESUS et al., 2009; MENDES et al., 2015; RODRIGUES et al., 2013) both tend to have greater "local" (alpha) diversity in modified environments than in undisturbed forests. On the other hand, after converting forest to pasture in the Western Amazonia, decreases in the "regional" diversity (beta diversity) of bacteria were noticed (RODRIGUES et al., 2013), indicating that the total diversity of soil microorganisms can be reduced by land-use intensification, despite the greater local diversity. This biotic homogenization of soil bacteria could compromise the stability of the ecosystem (NAEEM; LI, 1997), especially when longer time scales are considered (HOOPER et al., 2005). However, subsequent studies found different results for regional diversity, finding higher values in more anthropized systems, such as pastures and mechanized agriculture (DE CARVALHO et al., 2016). In a recent meta-analysis, the authors highlighted that the alpha diversity is consistently in land uses under intensification than in natural ecosystems. However, with respect to beta diversity, they elucidated that this measure remains a knowledge gap due to either inconsistencies in measurement methods or the lack thereof, requiring the development of more accurate tools to analyze this factor at larger landscape scales (PETERSEN; MEYER; BOHANNAN, 2019).

2.3 Clinical Aspects of Cattle Periodontitis ("cara-inchada dos bovinos – CIb" in Portuguese) and Associations with Disruption of Ecological Stability

Periodontitis is characterized by periodontal pocket formations, gum recession, loss of clinical attachment, and mobility, culminating in tooth loss. (BORSANELLI et al., 2018; DÖBEREINER et al., 2000). Initially, the disease was described as associated with the pasture formation in extensive areas of southeastern, central-western and northern Brazil (DÖBEREINER; DUTRA; ROSA, 2004), occurring in high prevalence after removal of the original vegetation to form pastures, pasture reform or when cattle in early teething stage were fed with forages grown in endemic areas (DÖBEREINER et al., 2000; DUTRA; MATSUMOTO; DÖBEREINER, 1993).

Dutra, Botteon and Döbereiner (2000) performed a longitudinal bacteriological study of periodontal lesions in calves transferred to a disease-free area and observed a modification of the oral microbiota after clinical remission of the disease ("clinical cure"). In peridentary pouches, 71.3% of the total microbiota that could be grown anaerobically in specific culture medium were black pigmented *Bacteroides*. After transfer to the area with no history of the disease, the relative abundance of these bacteria was decreased to 1.7%. These results supported the evidence that periodontitis is conducted as a multifactorial infectious disease.

Generally neglected in animal production, cattle periodontitis has a purulent, chronic characteristic, and its infectious process is progressive, causing cumulative changes throughout the bovine's life. For the establishment of periodontopathogens, they need to be able to colonize the subgingival sulcus and produce virulence factors that directly damage the host tissues (POPOVA; DOSSEVA-PANOVA; PANOV, 2013). This requires adherence of the pathogen to available surfaces, with subsequent multiplication and competition with other organisms, in addition to establishing forms of protection against host defense mechanisms (HAFFAJEE et al., 2008). Although its structure is still poorly understood, dental biofilm is reported to form complex structures adhering to tooth enamel. In diseased animals, the chemical structure of the biofilm

appears to contain higher Fe and Mn levels, and there are also other cocci and *bacilli* among the microbiota on the supragingival calculus (SARAIVA et al., 2020). When in homeostasis, the biofilm helps maintain oral health, and when in dysbiosis, it can act as an initiator of infectious processes (SCANNAPIECO; DONGARI-BAGTZOGLOU, 2021). It is also reported that although some taxa are commonly found, as such as *Prevotella, Fusobacterium, and Porphyromonas*, microbial profiles may differ according to the clinical condition of the animals (BORSANELLI et al., 2018). Moreover, animals affected by periodontitis present a higher ecological diversity in their microbial composition, being correlated with the dysbiosis in the oral microbiota (BORSANELLI et al., 2018), which is in line with the mechanisms currently described for periodontitis in humans (KUMAR, 2021; SCANNAPIECO; DONGARI-BAGTZOGLOU, 2021).

Previous studies also reported that streptomycin produced by soil streptomycetes, when ingested with the forage leaves would be one of the main causes of the development of cattle periodontitis (GRASSMANN et al., 1997; KOPP et al., 1996). Based on "*in vitro*"-assays, it was confirmed that soil streptomycin significantly increased up to 10-fold the adherence of bacteria associated with periodontal disease to host gingival epithelial cells, suggesting that antibiotics from this group play an important role in the pathogenesis of this multifactorial infectious disease (DÖBEREINER et al., 2000; GRASSMANN et al., 1997; KOPP et al., 1996). Studies have also reported that bacterial biofilm formation is enhanced under sub-inhibitory antibiotics doses, including streptomycin, which increase their resistance to environmental stressors in comparison to their free-living/planktonic counterparts (KAPLAN, 2011; KUMAR; TING, 2016).

Recently, Ramos et al. (2019) observed that the administration of virginiamycin significantly reduces the occurrence of less severe periodontal lesions such as gingivitis and necrotizing gingivitis, being a tool for preventive herd management. However, despite the advent of antibiotics for the recovery of sick cattle, the mechanisms that lead to the onset of the disease have not been completely clarified, with the occurrence of sporadic outbreaks of periodontitis to this day.

2.4 Application of "Omic" Tools to Tackle Ecological Issues

The next-generation sequencing (NGS) technology have allowed sequencing of a large scale and speed and can generate billions of readings at costs about 10,000 times lower than first generation of DNA sequencing. The application of NGS for the study of microbial communities is usually done by metagenomics or by the amplification of specific genes which bring some information about the taxonomic or functional constitution of the community (THOMAS; GILBERT; MEYER, 2012). The first method is based on sequencing whole metagenome of multiple organisms simultaneously, while the second approach is based on polymerase chain reaction (PCR) sequencing amplifications.

Advancing in "*omic*" techniques, drastic improvements are taking place in the field of microbial ecology research, also accompanied by a great reduction in time and resources spent. It have allowed a more comprehensive analysis of microbial communities, giving access to non-cultivated organisms, which represent the majority (~ 99%) of all microorganisms (XU; GUNSOLLEY, 2014). The traditional method of DNA sequencing is only able to separate species individually and, therefore, is unsuitable for processing complex environmental samples, especially for large-scale studies. Environmental samples usually contain hundreds or thousands of individuals in the DNA mixtures. Although conventional sequencing has provided the most efficient method for the development of large reference libraries for 'DNA barcoding', the number

of individuals in an environmental sample is beyond the reach of its capacity (SHOKRALLA et al., 2012). The recovery of DNA sequences from the thousands of species present in an environmental sample requires the ability to read multiple DNA samples in parallel; something that NGS work effectively and at ever lower costs. Furthermore, advanced computational methods have bring the possibility to infer measures of biodiversity over time and space, annotating and grouping DNA sequences with a combination of attribution and phylogenetic techniques (SHOKRALLA et al., 2012). The recent growth, both in number and breadth of studies using NGS platforms, demonstrates a paradigm shift in ecological research towards the use of high volumes of sequence data (SHOKRALLA et al., 2012).

2.4.1 Analysis of marker-gene data: amplicon single variants (ASVs) replacing operational taxonomic units (OTUs)

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Errors in Illumina-sequenced amplicon data are currently addressed by quality filtering and the construction of operational taxonomic units (OTUs): clusters of sequences that differ by less than a fixed dissimilarity threshold, commonly 3% (EDGAR, 2013; SCHLOSS et al., 2009). ASV methods infer the biological sequences in the sample prior to the introduction of amplification and sequencing errors and distinguish sequence variants differing by as little as one nucleotide (CALLAHAN; MCMURDIE; HOLMES, 2017). Clustering similar sequences reduces the error rate due to misdetection of biological variation, but in that case OTUs underutilize the quality of modern sequencing, not handling the possibility of resolving the fine-scale variation (ROSEN et al., 2012). According to Callahan et al. (2016), fine-scale variation can be informative about ecological niches (EREN et al., 2015), temporal dynamics (TIKHONOV; LEACH; WINGREEN, 2015), and population structure (ROSEN et al., 2012). ASVs has shown capabilities as good or better than OTUs methods by more accurately discriminating the ecological patterns of data sets (CALLAHAN et al., 2016; PARADA; NEEDHAM; FUHRMAN, 2016). Nevertheless, ASVs still has limitations in cases where the same genome has multiple sequence variant due to multiple copies of the target genetic locus. Although it is a 'resolution of exact sequence variants', it does not eliminate the limitations inherent in representing complex biological organisms by a short genetic barcode (CALLAHAN; MCMURDIE; HOLMES, 2017). To handle with ASVs, a model-based approach named Divisive Amplicon Denoising Algorithm (DADA) was developed initially for 454-sequenced amplicon data, a further updated for Illumina sequencing, then called DADA2 (https://github.com/benjjneb/dada2). Briefly, the DADA2 algorithm uses a statistical model of the amplicon sequencing error process to identify sequences that are repeatedly observed too many times to be consistent with being generated by amplicon sequencing errors, and thus must represent distinct true sequences present in the sample (CALLAHAN; MCMURDIE; HOLMES, 2019). For example, in a study comparing 3 different approaches, Callahan et al. (2017) found that DADA2 was able to distinguish *Staphylococcus aureus* and *Staphylococcus epidermidis* while Mothur and UPARSE methods merged these species into a single OTU. Based on DADA2 developers, replacing OTUs with ASVs makes marker-gene sequencing more precise, reusable, reproducible and comprehensive.

3. CHAPTER I

SOIL TYPE DETERMINES THE MAGNITUDE OF SOIL FERTILITY CHANGES BY FOREST-TO-PASTURE CONVERSION IN WESTERN AMAZONIA

ROCHA, F. I.; JESUS, E. C.; TEIXEIRA, W. G.; LUMBRERAS, J. F.; CLEMENTE, E. P.; MOTTA, P. E. F.; BORSANELLI, A. C.; DUTRA, I. S.; OLIVEIRA, A. P. Soil type determines the magnitude of soil fertility changes by forest-to-pasture conversion in Western Amazonia. **Science of the Total Environment**, v. 856 (Part I), 2022. https://doi.org/10.1016/j.scitotenv.2022.158955

3.1 RESUMO

A conversão da floresta tropical em pecuária extensiva tornou-se uma enorme preocupação ambiental na Amazônia brasileira. A remoção da floresta primária altera os componentes edáficos, afetando consequentemente a manutenção dos serviços ambientais. A maioria dos estudos sobre mudança de uso da terra na Amazônia abrangeram uma baixa heterogeneidade de classes de solo, limitando a compreensão de como os diferentes tipos de solos reagem aos impactos da mudança do sistema de uso da terra. Portanto, o principal objetivo deste estudo foi caracterizar como a conversão de florestas tropicais em pastagens afeta os atributos edáficos em diferentes classes de solo. Foram amostrados 13 sítios, entre florestas, pastagens recentes (≤ 7 anos), e pastagens antigas (≥ 10 anos), sobre Argissolos, Latossolos, Plintossolos, e Luvissolos, ao longo dos estados do Acre e Amazonas. Os solos coletados foram caracterizados por parâmetros químicos e físicos e classificados taxonomicamente. Além disso, testamos a sensibilidade dos filos bacterianos *Actinobacteria* e *Proteobacteria* em detectar transformações no ambiente do solo, com base no seu habitat ecológico. A análise de componentes principais evidenciou o gradiente inter-regional da fertilidade do solo, agrupando os sistemas avaliados principalmente pelo tipo de solo e uso da terra ao invés da distância geográfica entre localidades. Variáveis do solo como a soma de bases (SB), Ca+Mg, saturação de bases, saturação por alumínio e pH foram consistentemente afetadas pela conversão do uso da terra, com efeitos pronunciados em solos mais intemperizados. Contudo, a SB foi a única variável que apresentou efeitos estatisticamente significativos entre os locais de estudo, sendo um indicador capaz de expressar o efeito da conversão floresta-pastagem em diferentes tipos de solo. Finalmente, a razão *Actinobacteria:Proteobacteria* foi também sensível para refletir os efeitos da conversão floresta-pastagem nos atributos do solo, com uma relação mais elevada nos sistemas de pastagem, bem como positivamente correlacionada com o pH do solo. Os resultados demonstraram consistentemente que a conversão floresta-pastagem leva a fortes alterações no ambiente do solo, com intensidades variáveis dependendo da classe.

Palavras-chave: Pedodiversidade. Floresta amazônica. Microbiota edáfica. Mudança no uso da terra.

3.2 ABSTRACT

Converting the rainforest to extensive cattle ranching became a huge environmental concern in Brazilian Amazonia. Removing the pristine forest changes the edaphic components, consequently, affecting the maintenance of environmental services. Most of the surveys in Amazonia was carried out in a short range of soil heterogeneity, limiting the comprehension of how different types of soils react to the impacts of land-use system change. Therefore, the main goal of this study was to characterize how the conversion of tropical rainforest to pasture affects soil attributes across different soil classes. We sampled 13 sites, amongst forest, recent pasture (≤ 7-year-old), and old pasture (≥ 10-year-old), on Acrisols, Ferralsols, Plinthosols, and Luvisols, across the states of Acre and Amazonas, in the Brazilian Amazon region. Soils characterized for chemical and physical parameters and classified taxonomically. Furthermore, we tested the sensibility of the phyla *Actinobacteria* and *Proteobacteria* to detect transformations in the soil environment, based in their ecological habitat. Principal component analysis evidenced the inter-regional gradient of soil fertility, clustering soil mostly by their type and associated land-use instead of the spatial distance. Soil variables such as sum of bases (SB), Ca+Mg, base saturation, aluminum saturation, and pH were consistently affected by the land-use conversion, with pronounced effects on more oxidic soils. However, the SB was the only variable with increases statistically significant among the study sites, being able to detect the effect of anthropic land-use on greater coverage of soil classes. Finally, the *Actinobacteria:Proteobacteria* ratio was also sensitive to reflect the effects of forest-to-pasture conversion on soil attributes, with higher ratio in pasture systems, as well as positively correlated with the soil pH. Our results consistently shown that the forest-to-pasture conversion leads to strong alterations in the soil environment, with varying intensities depending on the soil class.

Keywords: Pedodiversity. Amazon rainforest. Soil microbiota. Land-use change.

3.3 INTRODUCTION

Soil as a fundamental natural resource performs key environmental, economic, and sociocultural functions and services (WUBIE; ASSEN, 2020). Nevertheless, the indiscriminate development of human activities has been altering the natural composition of this resource, with deleterious consequences at the local and global scales (DELGADO-BAQUERIZO et al., 2020). In this context, tropical forests have been intensely driven to a process of erosion of biodiversity, with hitherto little-known effects on the soil ecosystem (DE LIMA et al., 2020; PONGE, 2015). Extraction of high added-value wood and subsequent burning of remaining vegetation to introduce annual, perennial crops or pastures (MOREIRA; FAGERIA; GARCIA Y GARCIA, 2011) has been the main modifier of the Amazonian landscape since the 1960s (FEARNSIDE, 1996; NUMATA et al., 2007).

Understanding how land-use change affects the properties of different soil classes in biodiversity hotspots becomes vital to forecasting the consequences of the continuous conversion of forests to pastures (DIAS-FILHO; DAVIDSON; CARVALHO, 2001; NUMATA et al., 2007). Nonetheless, although the Amazonia encompasses a large diversity of soil types (QUESADA et al., 2010), studies have been carried out mainly on highly weathered soils such as Ferralsols and Acrisols, which cover most of the Amazon basin (DEMATTÊ; DEMATTÊ, 1996; SCHAEFER et al., 2017). To improve soil conservation practices and assist in decision making, it is necessary to increase information on how less studied soil types, such as naturally fertile Luvisols, besides the nutrient-poor Ferralsols, are affected by the land-use conversion, mainly which soil attributes are the most altered, in a broader conception.

The Amazon rainforest is one of the most oligotrophic forests in the world (BRAZ; FERNANDES; ALLEONI, 2013). That condition is driven mostly by geological aspects associated with the high weathering degree of the predominant soils and nutrient leaching (APRILE et al., 2013). However, the conversion of the primary rainforest to agricultural or pasture lands leads to a significant increase of pH and a reduction of exchangeable aluminum concentrations by the liming-effect of the deposited ashes from the combusted forest biomass (ALFAIA et al., 2004; MOREIRA; FAGERIA; GARCIA Y GARCIA, 2011). Moreover, alterations in the natural plant cover are also reported to change the activity, functions, and biomass of microbial groups, which are the main factors responsible for releasing nutrients into the soil (KASCHUK; ALBERTON; HUNGRIA, 2011), although it is generally time dependent (FARELLA et al., 2007), and variable by soil type (DE MORAES et al., 1996; MOREIRA; FAGERIA; GARCIA Y GARCIA, 2011).

Changes in the composition and structure of the soil microbial community tends to follow the gradient of transformations in attributes linked to soil fertility, mainly the soil pH (CLIVOT et al., 1996; KROEGER et al., 2018). Also, some bacterial groups such as Proteobacteria, for pristine forests, and Actinobacteria, for anthropized systems, have been reported to be able to reflect the alterations in the soil environment by land-use change (PETERSEN; MEYER; BOHANNAN, 2019). However, any application for its use as a microbiological indicator was not so far tested and proposed.

Therefore, the main goal of this study was to characterize how the conversion of tropical rainforest to pasture affects soil attributes across different soil types. It was hypothesized that the magnitude of transformations in the soil environment in response to land-use change varies due the soil type. Forests and pastures with different ages since their conversion were selected along a regional soil fertility gradient throughout the Brazilian Western Amazonia. Finally, as side evidence of how the forest-to-pasture conversion can affect edaphic variables, we addressed a

biological measurement to represent that scenario by taking advantage of the well-recognized characteristics of Actinobacteria and Proteobacteria as dominant phyla in copiotroph (i.e., high nutrient availability) and oligotroph (i.e., low nutrient availability) habitats, respectively (CLIVOT et al., 1996; FIERER et al., 2012). Additionally, we tested the use of the *Actinobacteria:Proteobacteria* ratio as a biotic indicator of transformations in the soil environment after the forest conversion.

3.4 MATERIAL AND METHODS

3.4.1 Sampling and experimental design

This study was carried out in the Brazilian Western Amazonia, within a geographical range of ± 800 km, which covers spots near the cities of Bujari, state of Acre, 9°49′22″S, 67°56′51″W, elevation 196 m), Boca do Acre (state of Amazonas, 8°44′26″S, 67°23′3″W, elevation 99 m) and Manicoré (state of Amazonas, 5°48′34″S, 61°18′2″W, elevation 32 m) (Figure 3). The climate of the region, characterized by tropical monsoon rain and a brief dry period between June and August, is classified as 'Am' according to the Köppen system. The annual average rainfall varies between 2200 and 2800 mm and the average annual temperature varies between 24 and 26°C (ALVARES, C. A., STAPE, J. L., SENTELHAS, P. C., GONÇALVES, J. D. M., & SPAROVEK, 2013). The parent materials for soils in the Western Amazonia region are mixed-textured Tertiary and Quaternary fluvial sediments of Andean origin (RODRIGUES, 1996).

The sites were selected based on their importance for tropical forest conservation as well as the rapid advance of livestock production, which has been reported as one of the main drivers of deforestation. Soil sampling was done in August 2017 following the experimental design used by the Sustainable Amazonia Network (GARDNER et al., 2013), with a total of 65 sampling points distributed among five forest areas and eight pasture areas, varying in age. The localities inserted in Manicoré/AM (MAN 1 and MAN 2) are areas of recent colonization (≤ 7 years since conversion), with no old pastures, thus, only results for new pastures were presented in this study. We took soil samples in linear transects that were 200 m in length, including five sample points separated by 50 m from each other varying according to the conditions of the sampling point. In each sample point, five subsampling (0-10 cm) was performed to generate a composite sample per point. For more details about landscape and sample conditions, see Table .

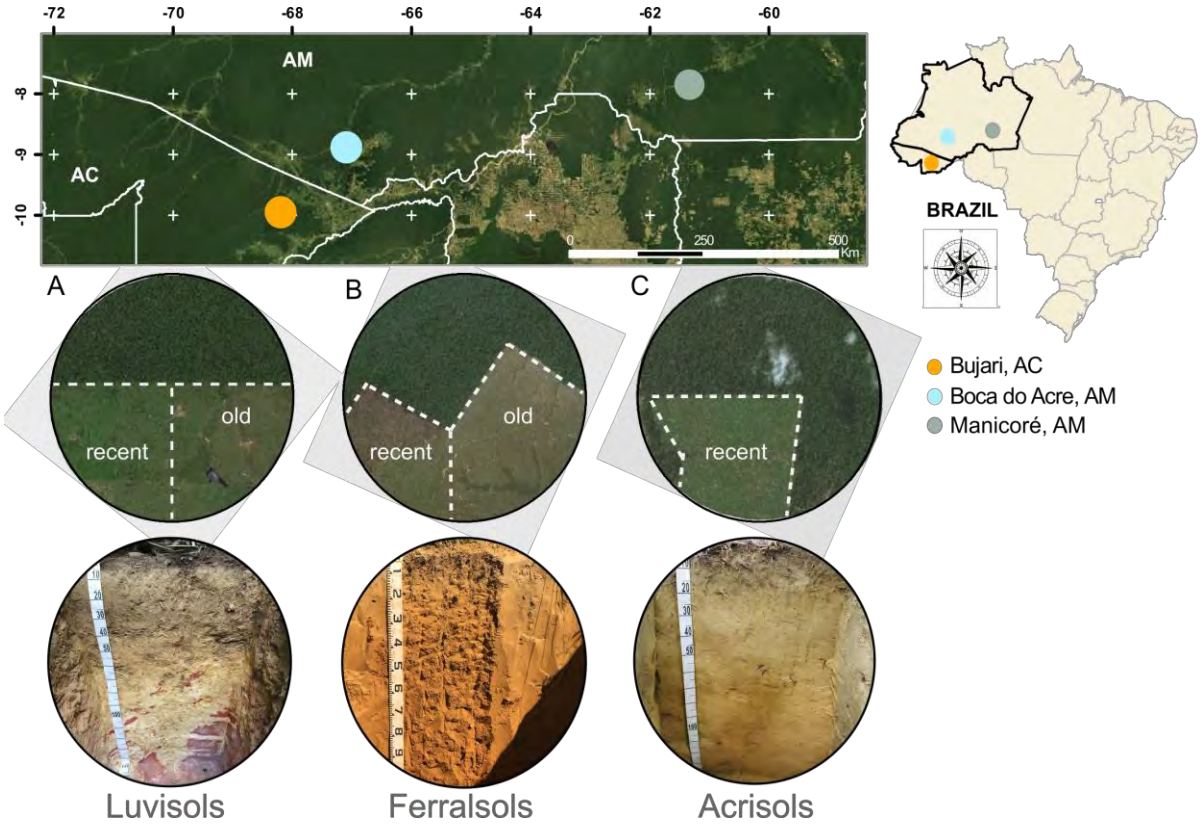

Figure 3. Study sites and sampling. Illustrative scheme of the study sites in the Brazilian Western Amazonia, with forest-to-pasture conversion areas and their predominant soil types shown below. A) Bujari (BUJ), state of Acre (AC); B) Boca do Acre (BAC), state of Amazonas (AM); and C) Manicoré (MAN) also in the state of Amazonas (AM). Satellite images provided by ESRI Service Layer.

3.4.2 Soil classification and characterization

The soils from all studied areas were classified, using one profile per land-use where morphological characterization and horizon soil sampling were carried out according to Santos et al. (2005). Soil physical attributes (particle size distribution and flocculation degree) were determined by the sedimentation method and reading by densimeter from the sample dispersion with 0.1 mol L–1 sodium hydroxide solution. The chemical analyses consisted of pH in water and KCl 1 mol L–1, determined potentiometrically, in the soil: 1:2.5 solution with 1 h of contact and agitation of the suspension at the time of reading. Exchangeable sodium and potassium (Na+ and K+) were extracted with HCl 0.5 mol L–1 + H2SO4 0.0125 mol L–1 (Mehlich–1), in the proportion of 1:10 and determined by photometry of flame emission. The measurement of exchangeable calcium and magnesium (Ca2+ and Mg2+) was performed by atomic absorption spectroscopy and exchangeable aluminum (Al3+) by titration after extraction with KCl 1 mol L–1 in the proportion of 1:10. The determination of potential acidity (H + Al) was carried out by titration after extraction with calcium acetate 0.5 mol L–1 in the proportion 1:10 and pH 7.0. The organic carbon was determined by titration of the remaining potassium dichromate with ammoniacal ferrous sulfate after the oxidation process. The calculation of derived correlations, i.e., sum of bases (SB = Ca2+ + Mg2+ + K+), base saturation index (BS% = 100 × SB/total cation exchange capacity (CEC)),

and Al saturation index (m% = (mmolc (Al_3^+) dm^{-3} × 100)/(mmolc (effective CEC) dm^{-3})), were also analyzed (TEIXEIRA et al., 2017) at the National Soil Research Center/ Embrapa Solos, Brazil.

The soil profiles were classified following the criterion of the Brazilian System of Soil Classification (DOS SANTOS et al., 2018) and World Soil Reference Base (WRB, 2015).

3.4.3 DNA extraction, high-throughput sequencing, and data processing

To access the relative abundance of phyla Actinobacteria and Proteobacteria, we extracted soil DNA using the standard DNeasy PowerSoil kit protocol (MO BIO Laboratories Inc.). Amplification of the 16S rRNA gene for soil samples was performed using barcoding DNA (CAPORASO et al., 2012). PCR products were purified and subjected to library preparation and sequencing with Illumina MiSeq technology following the Earth Microbiome Project protocol at the Argonne National Lab Core Sequencing Facility, USA. The 16S rRNA sequence data were further processed, aligned, and categorized using the DADA2 microbiome pipeline (https://github.com/benjjneb/dada2) by recommended parameters with quality filtering of sequence length over 250 base pairs (CALLAHAN et al., 2016). Further, the taxonomy was assigned for each ASV assessing the Silva taxonomic training (database v132) (QUAST et al., 2012). For details about next-generation sequencing parameters and bioinformatic pipelines, see Rocha et al. (2021). R packages 'dada2' v.1.14.0 (CALLAHAN et al., 2016) and 'decipher' v.2.14. (WRIGHT; YILMAZ; NOGUERA, 2012) were used in the R 3.6.1 environment (R Team, 2018).

3.4.4 Statistical analysis

A principal component analysis (PCA) on the correlation matrix was used to obtain a subset of the most important soil variables based on their component loadings (i.e., correlation between a principal component and the variable). Firstly, we performed a general PCA using all sample points and measured variables, to better obtain the dissimilarity between the study locations, based on their Euclidean distance. After that, all the variables with a contribution larger than the cutoff of 3.85% (i.e., 100 x (1/26 soil variables)) were selected. The contribution of a variable for a given PC was obtained by the ratio of the squared component loading of the variable by the eigenvalue associated with the PC, following Abdi and Williams (2010). All selected variables (highest PC1 contributions) of each study location were merged into a single subset to be further used in the two-way ANOVA for comparing both inter-regional (among sites), and intra-regional (between land-uses within each site). The PCAs were conducted using 'factoextra' v.1.0.7 R package (KASSAMBARA; MUNDT, 2018). A heatmap was used to visualize possible clusters among samples within each factor (i.e., site, land-use, and soil classes) and measured variables, using 'pheatmap' v.1.0.12 R package. In order to test their use as an ecological indicator of land-use change on soil attributes, relative abundances of *Actinobacteria* and *Proteobacteria* were compared using STAMP v.3.0 (PARKS et al., 2014). The q-values were calculated using two-sided Welch's t-test with Benjamini–Hochberg false discovery rate corrections (BENJAMINI; HOCHBERG, 1995). The correlation between *Actinobacteria* and *Proteobacteria*, as well as *Actinobacteria:Proteobacteria* ratio and soil pH was tested by the Spearman's rho test. Depending on the relevance of the analysis, pastures were categorized into recent and old in order to verify the temporal effect on soil attributes.

3.5 RESULTS AND DISCUSSION

3.5.1 Natural and anthropogenic factors build the inter and intra-regional gradient of soil fertility

After a principal component analysis including all studied sites (called here inter-regional PCA), a subset of fifteen variables was extracted based on their higher contribution values, pointing out dissimilarities in the soil physical and chemical properties, which explained 59.4% of total variability along the first two axes (Figure 4A). Furthermore, a PCA was carried out for each one of the studied locations (called here intra-regional PCAs), and those variables with the greatest contribution in each location were merged into a final subset of twenty soil variables that better represent the deforestation effect on the soil environment (see factor loadings for PC1 and PC2 on Table).

The inter-regional PCA ordered BUJ separately from BAC and MAN despite its geographical proximity to BAC, showing that the soil pedogenetic characteristics had a greater influence than land-use on the fertility of the soil superficial layer in a larger comparison scale. The PC1 scores of BUJ differed statistically in comparison to the scores from the other localities (Figure 5A; $\chi 2 = 37.59$, $p < 0.001$), being positively correlated with high values of total sum of bases (Figure 5B; Radj = 86%, $p < 0.001$). Nevertheless, a clear land-use effect could be observed within each of the studied locality, with a higher chemical fertility in pasture soils (Figure 5A), which has been demonstrated by other studies throughout the Amazon region (BRAZ; FERNANDES; ALLEONI, 2013; MACHADO et al., 2017). A higher dissimilarity between pastures and forests of low-fertility soils from BAC and MAN has also been observed, showing that the dissimilarity between land-uses within each location depends on the predominant soil type. Forest soils in BUJ have a higher natural fertility, which shows the clear influence of natural fertile Luvisols. Soils of the state of Acre were formed predominantly from the weathering of sedimentary rocks generated by the Andean orogeny and the sediment flux into lowland (QUESADA et al., 2011). Specifically, the Luvisols found in BUJ are a patch of naturally eutrophic soils (BERNINI et al., 2013). BAC and MAN are predominantly covered with high weathered soils, such as Acrisols and Ferralsols, developed from sandstones and claystones, and mainly formed in remnants of ferralitic and convex plateaus (SHINZATO; TEIXEIRA; DANTAS, 2015; SOUZA et al., 2018). Thus, the soil fertility reflects both the inherent pedogenetic characteristics, as well as the processes mediated by land-use conversion, which increases the dissimilarity between variables related to soil acidity (i.e., H+, H+Al, m%, Al) and those related to high soil fertility (i.e., pH, BS%, SB, T-CEC).

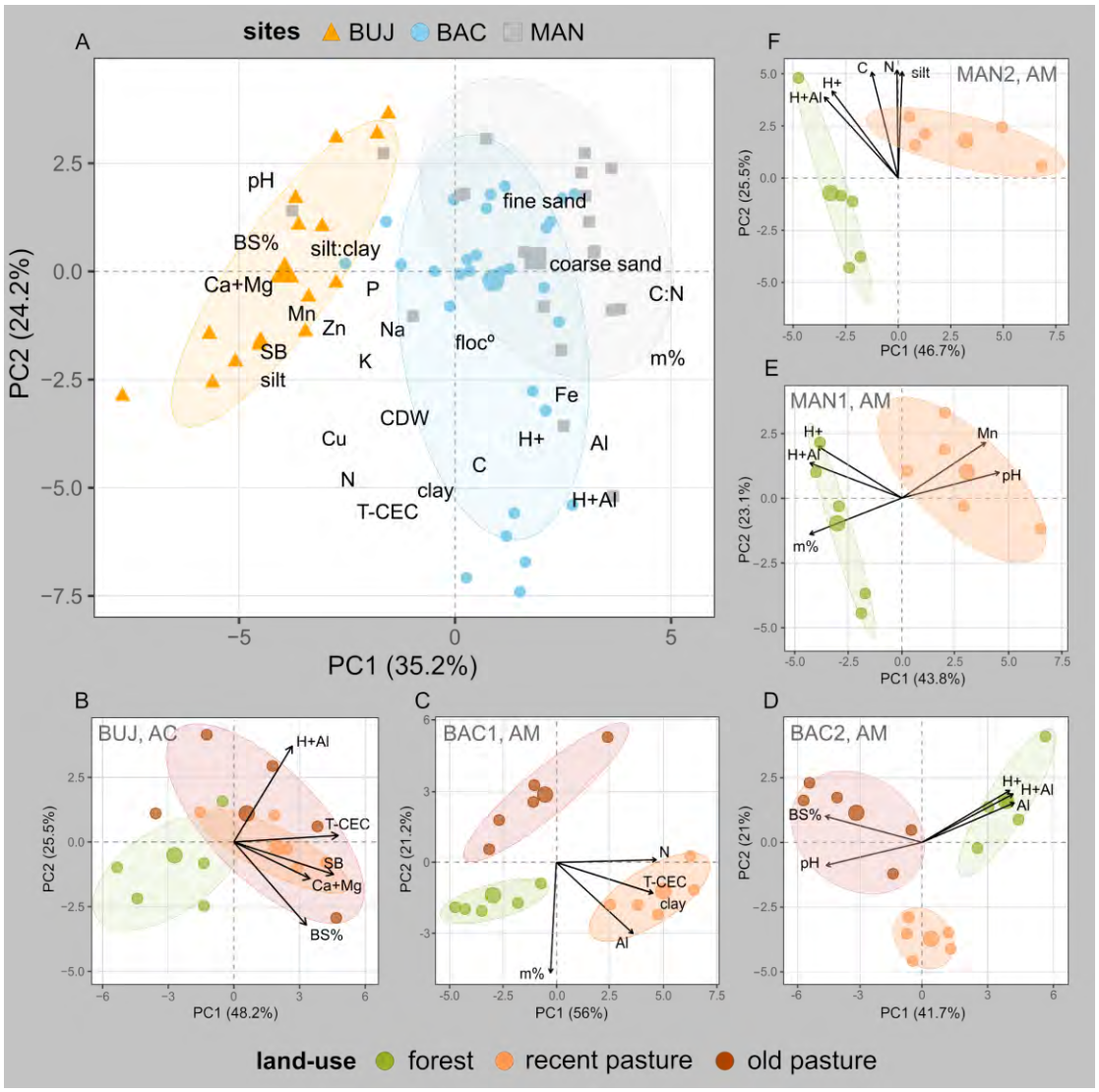

Figure 4. Ordination of soil variables across Amazonian sites and their altered land uses by the forest-to-pasture conversion. A) Principal component analysis (PCA) including all observations amongst study sites in the Brazilian Western Amazonia and (B - F) individual PCAs for each one of the study localities displaying the five most important variables based on their contribution value. BUJ: Bujari / state of Acre, BAC: Boca do Acre / state of Amazonas, including BAC1 and BAC2 localities, MAN: Manicoré/ state of Amazonas, including MAN1 and MAN2 localities.

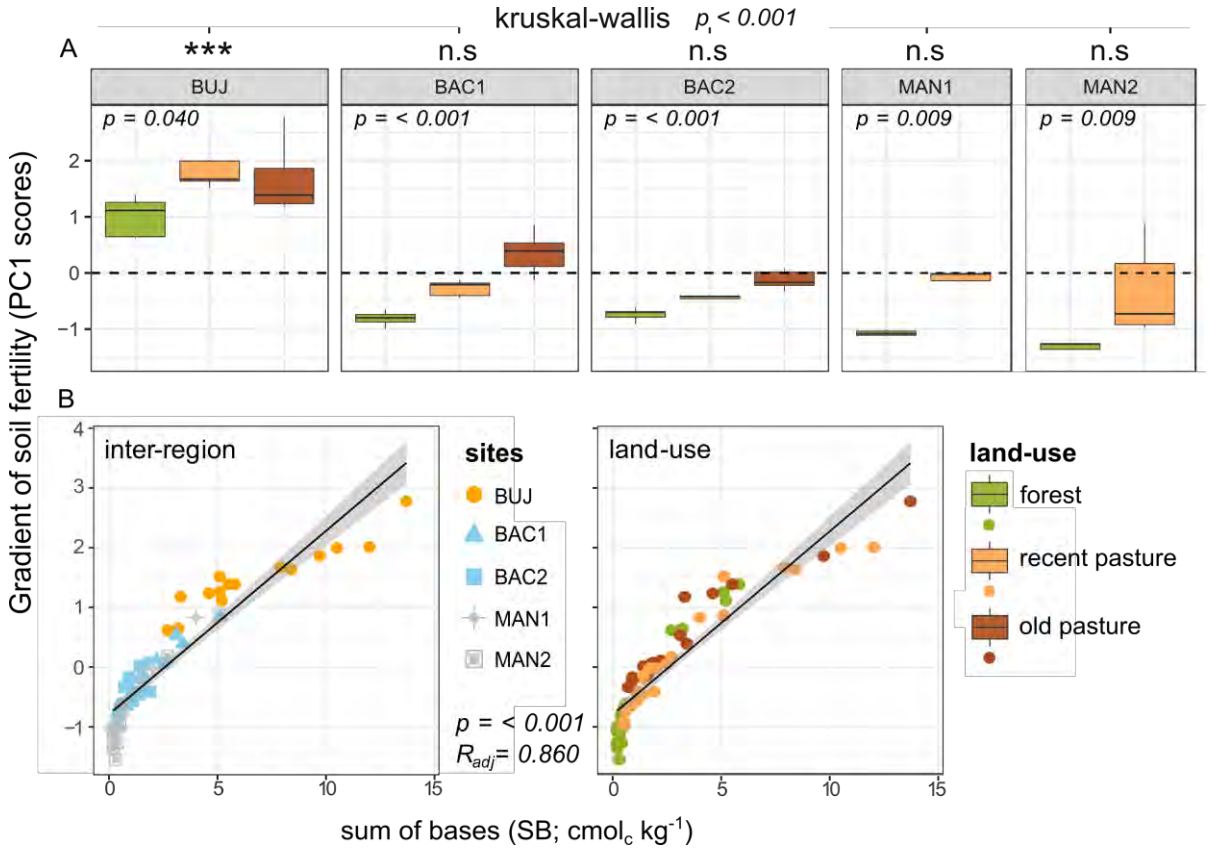

Figure 5. Inter and intra-regional trends in the gradient of soil fertility across land-use change profiles. A) Inter-regional (between sites) and land-use systems (within sites) comparisons; B) linear correlation between PC1 scores for the overall PCA and total sum of bases (SB) (equation: y = - 0.79 + 0.31x); localities were colored based in their site/municipality (i.e., orange: BUJ, blue: BAC, gray: MAN). Significant inter-regional and intra-regional/land-uses differences were determined by two-way ANOVA ($p < 0.05$); Permutational pairwise t-test was performed to detect the differences between study localities; (***) significant at $p < 0.001$, n.s. non-significant. The fitted values for each model are represented by the black line and their standard errors are indicated by the shaded area. BUJ: Bujari / state of Acre, BAC: Boca do Acre / state of Amazonas, including BAC1 and BAC2 localities, MAN: Manicoré / state of Amazonas, including MAN1 and MAN2 localities.

The evaluated systems are displayed into two major groups as showed by a hierarchical dendrogram based on the fifteen most important soil variables (Figure 6). The first group was correlated with variables linked to high soil fertility (high pH, Mn, BS%, SB, Ca+Mg), clustering the BUJ's land-uses and their respective soil types (i.e., Luvisol, Stagnic Plinthosol and Xanthic Acrisol or Luvissolo Háplico, Plintossolo Argilúvico and Argissolo Amarelo, respectively, based on Brazilian Soil Classification System). The second cluster has an intermediate subgroup comprising both a pasture and a forest of BAC, which were more positively correlated with clay, C, N, and T-CEC, as well as the soil acidity variables (H+, H+Al, Al, and m%). The largest subgroup includes land uses from BAC and MAN, where the forests are positively correlated with the soil acidity variables, as well as the C:N ratio, which were more pronounced in those systems. In turn, pronounced influence of the land management decreased the content of soil acidity

variables on pastures, although those systems are on weathered soils of the Amazonian region, as pointed out above.

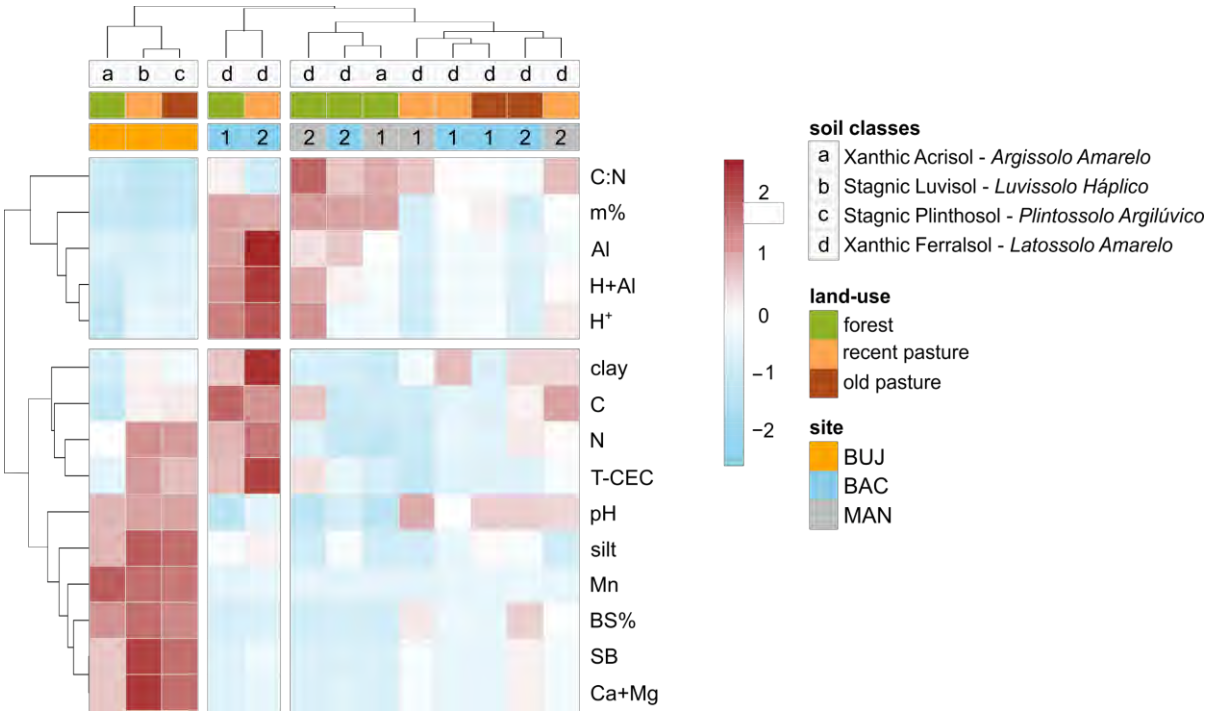

Figure 6. Correlation heatmap of soil variables. Horizontal dendrograms gather correlated soil classes, sites, and land-uses, based on the soil variables clustering in the vertical axis. BUJ: Bujari/ state of Acre, BAC: Boca do Acre/ state of Amazonas, including BAC1 and BAC2 localities, MAN: Manicoré/ state of Amazonas, including MAN1 and MAN2 localities.

Figure **7** presents the two-way ANOVA between forests and pastures for those twenty soil variables extracted with the contribution criterion after the PCAs performed for each study site (see subsection 3.4.4 in methods). The results show that only the Ca+Mg and sum of bases (SB) differ between forests and pastures in all study locations. Soil pH, aluminum saturation (m%), and base saturation (BS%) differed statistically between land-uses in BAC and MAN, but not in BUJ. Especially, the BUJ land uses showed the lowest m% values among the studied locations. BUJ forest was 79% lower than the average m% of forests in MAN (second lowest m% amongst forests), and the pasture was 12% lower than MAN pastures. The average values and deviations of the soil physical and chemical variables for each land-use evidence how soil formation processes and, consequently, the different soil types are important issues to be considered. Despite the high similarity between BUJ's land-uses regarding soil variables linked to soil fertility indices (i.e., pH, m%, and BS%), this does not indicate that the effect of converting forest to pasture is unable of exerting drastic changes in edaphic properties. Instead, the results show only that these changes are less pronounced due to the soil type, which gives them greater buffering capacity and natural fertility. In that case, more sensitive indicator of land-use change must be settled to detect variations between soils under distinct land-uses but with high base saturation in the first horizon.

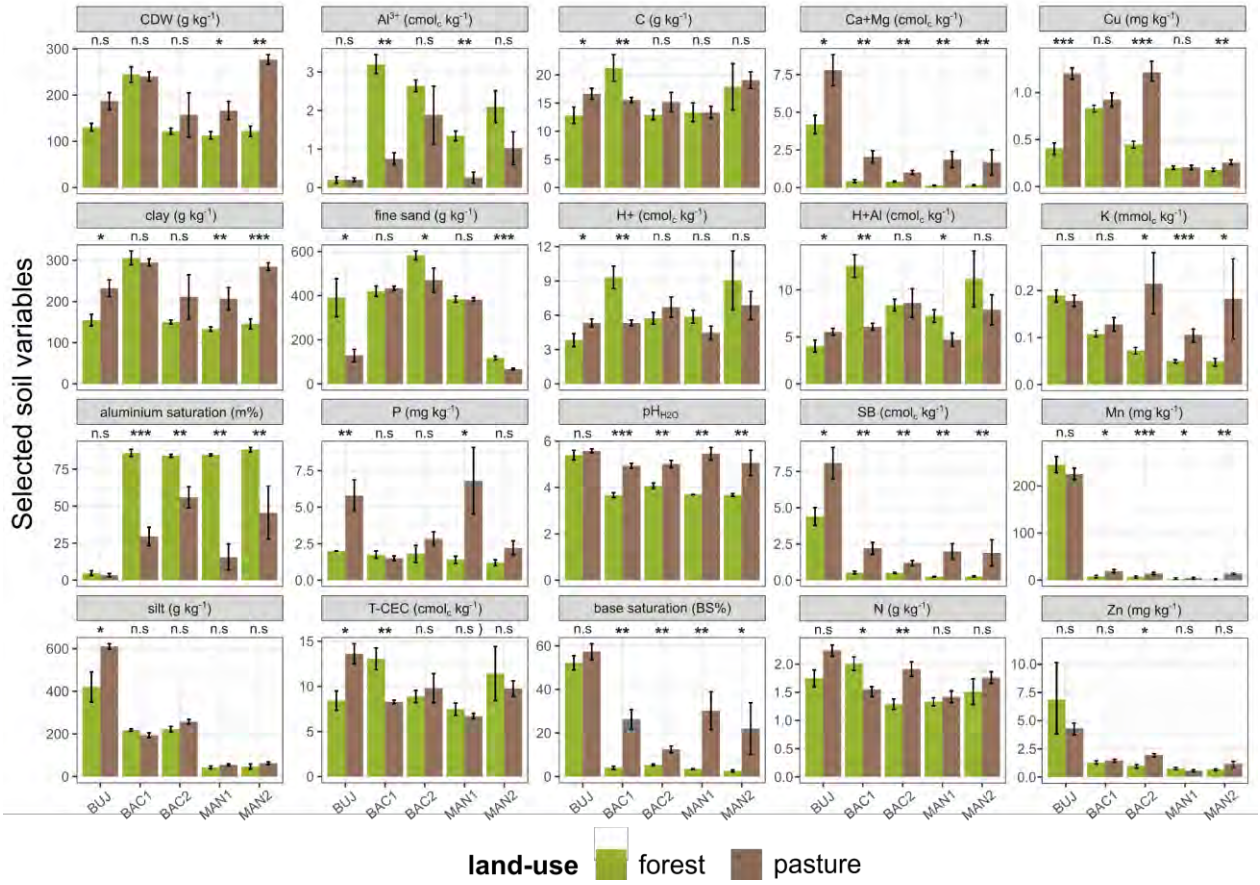

Figure 7. Bar plots for the statistical comparison between forests and pastures for selected soil variables. Soil variables (0-10 cm) were extracted as important in the principal component analysis among different study localities in Western Amazonia. Significant differences between each land-use were determined by two-way ANOVA ($p < 0.05$); (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. non-significant. Error bars indicate the ± standard error (SE) (n = 5). BUJ: Bujari/ state of Acre, BAC1 and BAC2: Boca do Acre / state of Amazonas, MAN1 and MAN2: Manicoré / state of Amazonas.

Although the soil phosphorus (P) is recognized for its low mobility, mostly in tropical soils, this variable was important to differentiate BUJ's land uses, being higher in pasture soils (forest: 2.0 mg kg-1, pasture: 5.8 ± 3.18 mg kg-1; $\chi 2 = 9.94$, $p < 0.01$). Moreover, we considered the standardized effect size of the comparison between forest and pasture for the indicators of soil fertility (i.e., H+Al, m%, pH, and SB) in each individual locality, as well as the overall effect among sites (Figure 8). The analysis evidenced that the m% is representative for most of the forest systems, whereas the soil pH and SB are highly representative for pastures. Sum of bases was the most sensitive variable to discriminate the effect of land-use change at all sites, which suggests it as a suitable indicator of these effects.

The forest-to-pasture conversion also results in soil chemistry transformations in depth (Figure 8). Characterizing soil profiles allows us to understand edaphic characteristics based on the description of each horizon, which surpasses the understanding of changes in the composition of soil variables only on the soil surface. Especially for m%, SB, and BS%, transformations between the relative comparison of horizons A and B were perceived among pasture soils, with a relative

higher proportion of the variables related to the increased exchangeable cations in the horizon A over B. The total and saturation levels for aluminum (Al and m%, respectively) evidenced that for BUJ, regardless of land-use, there is a considerable contrast between the two horizons, with values higher than 95% on B horizon. This reflects the aluminic character of most of the soils characterized in this study, corroborating previous reports for the state of Acre (LIPS; DUIVENVOORDEN, 1996). A relative decrease from 97% to 51% in m% between forest and pasture soil, and from approximately 80% to 40% for Al was observed for site MAN1. This is due to the direct effect of the transformations of the soil surface after conversion from forest to pasture and subsequent management of the extensive system for livestock production. Sharing the same trends as Müller et al. (2004), BRAZ et al. (2013), and Krainovic et al. (2020) our results did not clearly indicate a decrease in soil base saturation among pastures over time. Moreover, the Al saturation (m%) and exchangeable aluminum (H+Al) were lower in older pastures (Figure 9).

Numata et al. (2007) in the state of Rondônia, also in the Brazilian Western Amazonia, did not find significant influence of the land-use change on soil fertility when considering different pasture ages since the forest removal. Instead, the study also found that the magnitude of the effects varies significantly among soil types, with low amplitude in Luvisols. Contrary to previous studies (DE MORAES et al., 1996; MOREIRA et al., 2009) our results exhibit an overall standardized effect that reflects the increase in soil pH over time since forest conversion. Mostly, recently formed pastures with higher values for soil acidity variables partially disagree with the assumption that pastures are fragile to maintain soil fertility, due to little protection against laminar erosion, intensified by the effects of high precipitation volume characteristic of these regions, enhancing the nutrient depletion (LÜ et al., 2007). The changes in the soil environment after the conversion of forest-to-pasture through slash-and-burn is commonly accompanied by the release of basic cations previously stored in the forest biomass, which contribute to increase the soil pH and base saturation (BÉLIVEAU et al., 2015; COCHRANE; SANCHEZ, 1982). No differences were observed between old and recently formed pastures for any indicator of soil fertility of BUJ, which is also explained by the buffering effect of the soil, which reduces the intensity of chemical transformations in the topsoil.

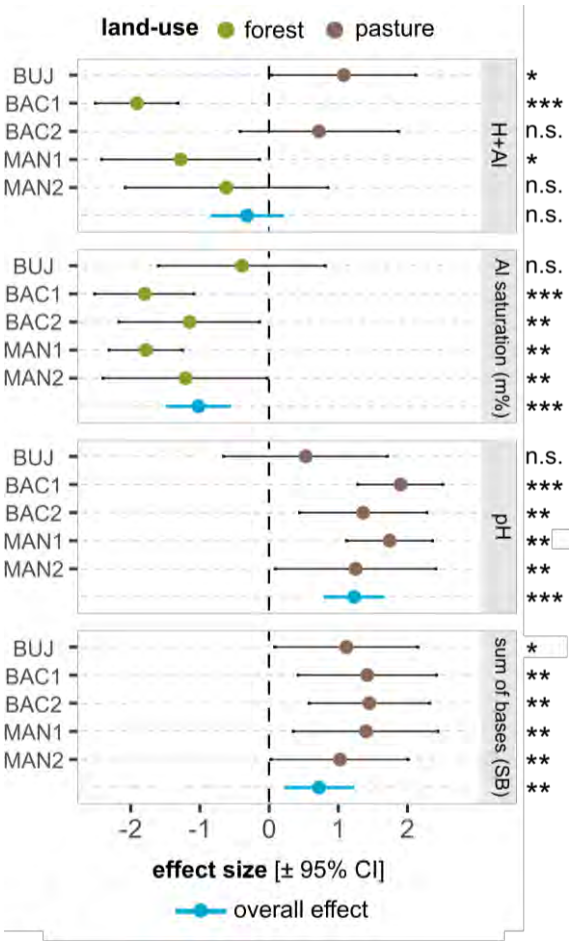

Figure 8. Standardized effect size of the forest-to-pasture conversion on soil variables. Coefficient estimates from linear models are plotted with 95% confidence intervals, where the dots represent the standardized effect size for the model between land-use and soil variables; The overall effect represent the magnitude of the effect considering all study sites in the model; Statistical differences were determined by two-way ANOVA ($p < 0.05$), (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. non-significant; BUJ: Bujari / state of Acre, BAC1 and BAC2: Boca do Acre / state of Amazonas, MAN1 and MAN2: Manicoré / state of Amazonas.

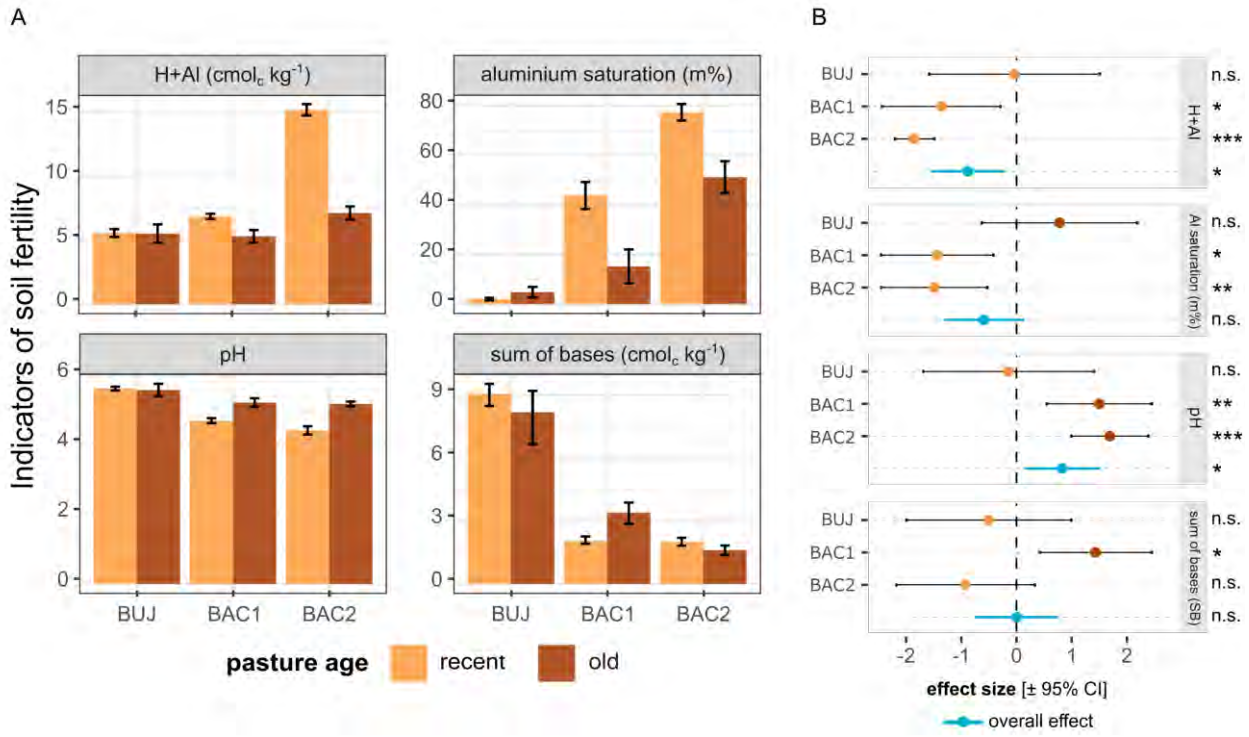

Figure 9. Influence of pasture age since forest conversion on soil fertility indicators in Amazonian localities. A) Bar plots between each pasture age and soil variables, error bars indicate the ± standard error (SE) (n = 5); B) Standardized effect size for the model between pasture age and soil variables, coefficient estimates from linear models are plotted with 95% confidence intervals; The overall effect represent the magnitude of the effect considering all study localities in the model; Statistical differences were determined by two-way ANOVA ($p < 0.05$), (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. non-significant; BUJ: Bujari/ state of Acre, BAC1 and BAC2: Boca do Acre/ state of Amazonas.

3.5.2 *Actinobacteria:Proteobacteria* ratio as a biotic indicator of the gradient of soil fertility

A general trend found among the study locations shows that the predominance of Proteobacteria in the forest system clearly reflects the natural conditions of its topsoil, despite the differences found between the soil attributes in the regions and soil types covered in this study (Figure 10). In turn, despite their highest relative abundance among the evaluated pastures, Actinobacteria did not differed statistically ($p > 0.05$) between land-uses of BAC2, MAN1, and MAN2.

Petersen et al. (2019) in a metanalysis that coupled several tropical systems under land-use change found that soil pH is a key variable to drive changes in the soil microbiota after forest-to-pasture conversion. Besides the increase on bacterial alpha diversity, they pointed out that the relative abundance of Actinobacteria increase in converted pastures soil, and that Proteobacteria is a representative group in tropical rainforests Overall, our results show that the relative abundance of Proteobacteria and Actinobacteria are negatively correlated (rho = - 44.2%, $p < 0.001$), with similar findings in most study locations. The discrepancy in the relative abundances found in the MAN2 site is likely reflecting some of its inherent characteristics, as seen in the

Figure **7**, since no statistical differences were found between its forest and pasture for the soil variables: C, H+, H+Al, P, T-CEC, N, and Zn. Moreover, the sandy texture associated with high levels of C:N ratio predominantly found in this locality can drive the reduced variability in the relative abundance of Actinobacteria between the land uses, since this bacterial group is commonly correlated with copiotroph environments (FIERER et al., 2012).

Nonetheless, the *Actinobacteria:Proteobacteria* ratio was sensible to representing the gradient of soil fertility across sites and land-uses for most of the evaluated localities (Figure 10), following a positive correlation with soil pH (rho = 46.9%, $p < 0.001$). Microbial communities are intrinsically shaped by the soil environment, especially the topsoil which encompasses the rhizosphere activity, complex biological interactions, organic matter decomposition, and food webs, which drives the soil chemical complex (SULEIMAN et al., 2013; TRIPATHI et al., 2018). Considering this, the use of the present biological indicator may be useful for microbial ecologists interested in mechanisms to measure the fingerprints of land-use changes on tropical soil environments.

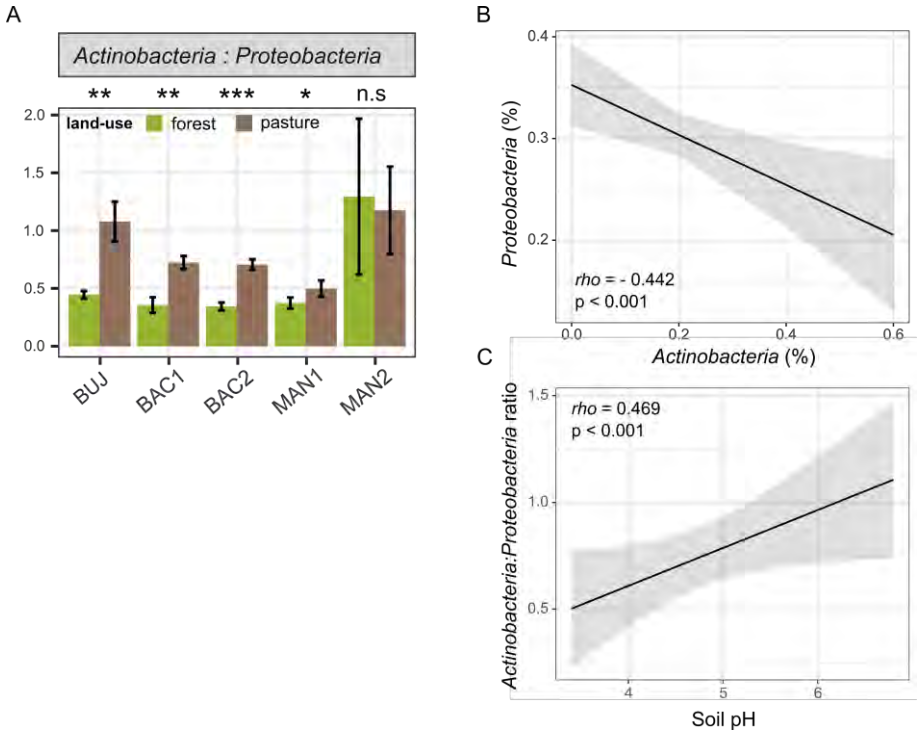

Figure 10. *Actinobacteria:Proteobacteria* ratio as a biological indicator of land-use conversion. A) Significant differences between each land-use were determined by two-way ANOVA ($p < 0.05$); (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. non-significant; Error bars indicate the ± standard error (SE) (n = 5). Spearman's rho correlation between B) Actinobacteria and Proteobacteria phyla, and C) *Actinobacteria:Proteobacteria* ratio and soil pH. The fitted values for each model are represented by the black line and their standard errors are indicated by the shaded area. BUJ: Bujari/ state of Acre, BAC1 and BAC2: Boca do Acre/ state of Amazonas, MAN1 and MAN2: Manicoré/ state of Amazonas.

3.6 CONCLUSIONS

This study showed that the intensity of transformations in the soil attributes by anthropic use depends mostly on the soil type, which regulates how wide the difference will be in the balance of variables related to base saturation and soil acidity among different natural and anthropic land uses, therefore, with considerable site-specific influence. Among the soil attributes, the sum of bases was the most sensitive to discriminate forest and pasture under the different soil types. The *Actinobacteria:Proteobacteria* ratio was sensitive in reflecting the gradient of soil fertility derived from the impact of land-use change on the soil environment, being a potential microbiological indicator of forest-to-pasture conversion in tropical ecosystems.

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4 CHAPTER II

LAND-USE SYSTEM AND FOREST FLOOR EXPLAIN PROKARYOTIC METACOMMUNITY STRUCTURING AND SPATIAL TURNOVER IN AMAZONIAN FOREST-TO-PASTURE CONVERSION AREAS

ROCHA, F. I.; RIBEIRO, T. G.; FONTES, M. A.; SCHWAB, S.; COELHO, M. R. R.; LUMBRERAS, J. F.; DA MOTTA, P. E. F.; TEIXEIRA W. G.; COLE, J.; BORSANELLI. A. C.; DUTRA, I. S.; HOWE A.; DE OLIVEIRA, A. P.; JESUS, E. C. Land-Use System and Forest Floor Explain Prokaryotic Metacommunity Structuring and Spatial Turnover in Amazonian Forest-to-Pasture Conversion Areas. **Frontiers in Microbiology**, v. 12, p. 909, 2021. doi: 10.3389/fmicb.2021.657508

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4.1 RESUMO

O avanço da pecuária extensiva é uma das principais ameaças à conservação da biodiversidade na Amazônia. A cobertura vegetal dominante tem um impacto drástico nas comunidades microbianas do solo, afetando sua composição, estrutura e serviços ecológicos. Aqui, exploramos as relações entre o uso da terra, tipos de solo e compartimentos do solo da floresta na estruturação da metacomunidade procariótica na Amazônia Ocidental. Amostras de solo foram coletadas em locais sob alta pressão antrópica e distribuídas ao longo de um gradiente de ± 800 km. Além disso, a serapilheira e uma camada de raiz, características do ambiente florestal, foram amostradas. O DNA foi extraído e a composição e estrutura da metacomunidade foram avaliadas por meio do sequenciamento do gene 16S rRNA. A metacomunidade procariótica do solo foi fortemente afetadas por pH, saturação de bases e alumínio, concentração de Ca + Mg, soma de bases e porcentagem de silte, devido ao manejo do uso da terra e diferenças naturais entre os tipos de solo. Maiores diversidades alfa, beta e gama foram observadas em locais com maior pH e fertilidade do solo, como solos de pastagem ou solos férteis do estado do Acre. Ao levar em consideração as comunidades da camada de serapilheira e da raiz, a diversidade beta foi significativamente maior no chão da floresta do que no solo de pastagem em todas as regiões de estudo. Nossos resultados mostram que a metacomunidade procariótica do chão da floresta realiza um *turnover* espacial até então subestimado para a escala regional de diversidade.

Palavras-chave: Amazônia. Floresta tropical. 16S rRNA. Sequenciamento de última geração. Biodiversidade microbiana. Mudança do uso da terra. Procariotos.

4.2 ABSTRACT

Advancing extensive cattle production is a major threat to biodiversity conservation in Amazonia. The dominant vegetation cover has a drastic impact on soil microbial communities, affecting their composition, structure, and ecological services. Herein, we explored relationships between land-use, soil types, and forest floor compartments on the prokaryotic metacommunity structuring in Western Amazonia. Soil samples were taken in sites under high anthropogenic pressure and distributed along a ± 800 km gradient. Additionally, the litter and a root layer, characteristic of the forest environment, were sampled. DNA was extracted, and metacommunity composition and structure were assessed through 16S rRNA gene sequencing. Prokaryotic metacommunities in the bulk soil were strongly affected by pH, base and aluminum saturation, Ca + Mg concentration, the sum of bases, and silt percentage, due to land-use management and natural differences among the soil types. Higher alpha, beta, and gamma diversities were observed in sites with higher soil pH and fertility, such as pasture soils or fertile soils of the state of Acre. When taking litter and root layer communities into account, the beta diversity was significantly higher in the forest floor than in pasture bulk soil for all study regions. Our results show that the forest floor's prokaryotic metacommunity performs a spatial turnover hitherto underestimated to the regional scale of diversity.

Keywords: Amazonia. Tropical rainforest. 16S rRNA. Next-generation-sequencing. Microbial biodiversity. Land-use change. Prokaryotes.

4.3 INTRODUCTION

Habitat fragmentation and land-use changes have led to an alarming and rapid decline of biodiversity in tropical ecosystems (NOBRE et al., 2016). Soil microbiomes, which are vital to ecosystem functioning and comprise a great capacity to reflect the impact of the land-use intensification on natural resources (BARNES et al., 2017), are one of the affected components of this biodiversity (APONTE; GARCÍA; MARAÑÓN, 2013; USHIO; KITAYAMA; BALSER, 2010). Consequently, it is crucial to understand how the conversion of tropical forests to other land-use systems affects edaphic microbiota, especially prokaryotes (HUG et al., 2016). Previous studies have identified a strong relationship between bacterial biodiversity, soil properties, and land-use systems in the Amazon rainforest (DE CARVALHO et al., 2016; JESUS et al., 2009; MENDES et al., 2015; NAVARRETE et al., 2015; PEDRINHO et al., 2019; RODRIGUES et al., 2013). These findings have shown that deforestation followed by the introduction of pastures and agricultural systems increase the alpha "local" diversity (average sample diversity) of soil bacteria, contrary to the previous expectation that bacterial diversity would be positively correlated with plant diversity (PROBER et al., 2015). Moreover, these studies have shown that the consequent increase in soil pH by the land-use conversion is one of the main abiotic factors shifting microbial community structure and diversity.

A still unresolved question is whether intensification of converted tropical ecosystems may contribute to soil microbial homogenization across space (PETERSEN; MEYER; BOHANNAN, 2019), declining the beta diversity (average dissimilarity in composition among sub-communities) (Anderson et al., 2006). Available studies suggest that, although land-use intensification tends to increase microbial alpha diversity, this effect does not persist on the beta diversity scale, possibly decreasing the gamma "regional" diversity (total observed diversity of all samples within) (WALTERS; MARTINY, 2020), and a decline in microbial turnover across space (GOSS-SOUZA et al., 2017; MENDES et al., 2015; RODRIGUES et al., 2013). However, contrasting results (DE CARVALHO et al., 2016; LEE-CRUZ et al., 2013) indicate higher components of diversity (alpha, beta, and gamma) over more intensive land uses due to the increased environmental heterogeneity, evidencing contrary trends to microbial homogenization after the land-use change.

Previous studies have been carried out with a low variety of soil types, which reduces the ability to predict different drivers in the structuring of microbial communities, besides being predominantly limited to the topsoil (i.e., borderline range between soil profile and its top organic layers). Nonetheless, organic horizons are known to sustain ecosystem functioning, especially in tropical forests (SAYER; TANNER, 2010) that predominantly grow on nutrient-poor soils (GRUBB, 1995). Some recent efforts have investigated how microbial communities in the litter interact with the soil microbiota (BUSCARDO et al., 2018; RITTER et al., 2018, 2020), but it is still unknown how microbial communities in the tropical forest floor (association between litter, root layer, and bulk soil) respond to regional scales of diversity. Moreover, clearing techniques traditionally used to remove the forest involve burning most of its biomass and are the principal deforestation method in Amazonia (BRANDO et al., 2020). Thus, filling this knowledge gap is essential to measure the effects of biodiversity loss in tropical rainforests.

In this study, we tackled how prokaryotic metacommunity (i.e., microbiota assemblies from spatially different sites) in the Western Amazonian forest floor contributes to spatial turnover and gamma "regional" diversity. We hypothesize that the lower alpha microbial diversity of the forest soil, reported in previous studies, is a sampling artifact caused by the non-inclusion of the forest floor as a whole, that is, by not taking into account its organic layers. We also hypothesized that

the beta and gamma diversities are higher in the forest floor's prokaryotic community than in the pasture bulk soil. We took advantage of a broad Amazonian pedodiversity, ranging from a patch of natural nutrient-rich soils in the state of Acre (e.g., Luvisols) to those with a high weathering degree in the state of Amazonas (e.g., Acrisols and Ferralsols) to test whether soil type rather than land-use history is a significant factor structuring prokaryotic metacommunity. To investigate these effects, we targeted the 16S rRNA gene using amplicon/barcode sequencing to assess microbiota in a geographic gradient that covers an extensive range of soils and landscapes in the Western Amazonia under the effects of recent forest-to-pasture conversion.

4.4 MATERIAL AND METHODS

4.4.1 Sampling and experimental design

This study was carried out in the Brazilian Western Amazonia, within a geographical range of ± 800 km, which covers spots near the cities of Bujari (state of Acre, 9°49′22″S, 67°56′51″W, elevation 196 m), Boca do Acre (state of Amazonas, 8°44′26″S, 67°23′3″W, elevation 99 m) and Manicoré (state of Amazonas, 5°48′34″S, 61°18′2″W, elevation 32 m) (Figure 3). The climate of the region, characterized by tropical monsoon rain and a brief dry period between June and August, is classified as 'Am' according to the Köppen system. The annual average rainfall varies between 2200 and 2800 mm, and the average annual temperature varies between 24 and 26°C (ALVARES, C. A., STAPE, J. L., SENTELHAS, P. C., GONÇALVES, J. D. M., & SPAROVEK, 2013). The parent materials for soils in the Western Amazon region are mixed-textured Tertiary and Quaternary fluvial sediments of Andean origin (RODRIGUES, 1996). The sites were selected based on their importance for tropical forest conservation and the rapid advance of livestock production, which has been reported as one of the main drivers of deforestation. Sampling took place in August 2017 following the Sustainable Amazonia Network's experimental design (GARDNER et al., 2013), with a total of 65 sampling points distributed among five forests and eight pasture areas. We used 200 m linear transects, including five sampling points equally spaced 50 m apart. Composite soil samples were collected at each sampling point for both molecular analysis and soil characterization. Three pooled subsamples formed each composite sample.

Traditional sampling for molecular microbial ecology studies usually removes the litter before sampling (DE CARVALHO et al., 2016; KHAN et al., 2019; MENDES et al., 2015; PEDRINHO et al., 2019). Nevertheless, when visiting our study sites, we observed that the forest floor has a root layer on top of the mineral soil core, which is intertwined with particulate organic matter and decomposed litter. This layer is thicker and has similar aspects to an H horizon (Figure 11) in some forests, such as in Manicoré/AM. For this reason, we stratified samples in the forest floor into litter (leaves, mostly), root layer, and the mineral bulk soil (soil A-horizon at a depth of 0-10 cm; hereafter bulk soil). Sampling was done at each point of the linear transect, also formed by three pooled subsamples. The forest root layer was involved by particulate organic matter, which was recovered by sieving (2 mm mesh) and used for DNA extraction. Only the bulk soil (0-10 cm) was sampled in pasture lands since no superficial root layer, nor a significant litter component existed in these systems. All material sampled for molecular analysis was immediately packed in sterile pouches and refrigerated at -80ºC in the shortest time possible.

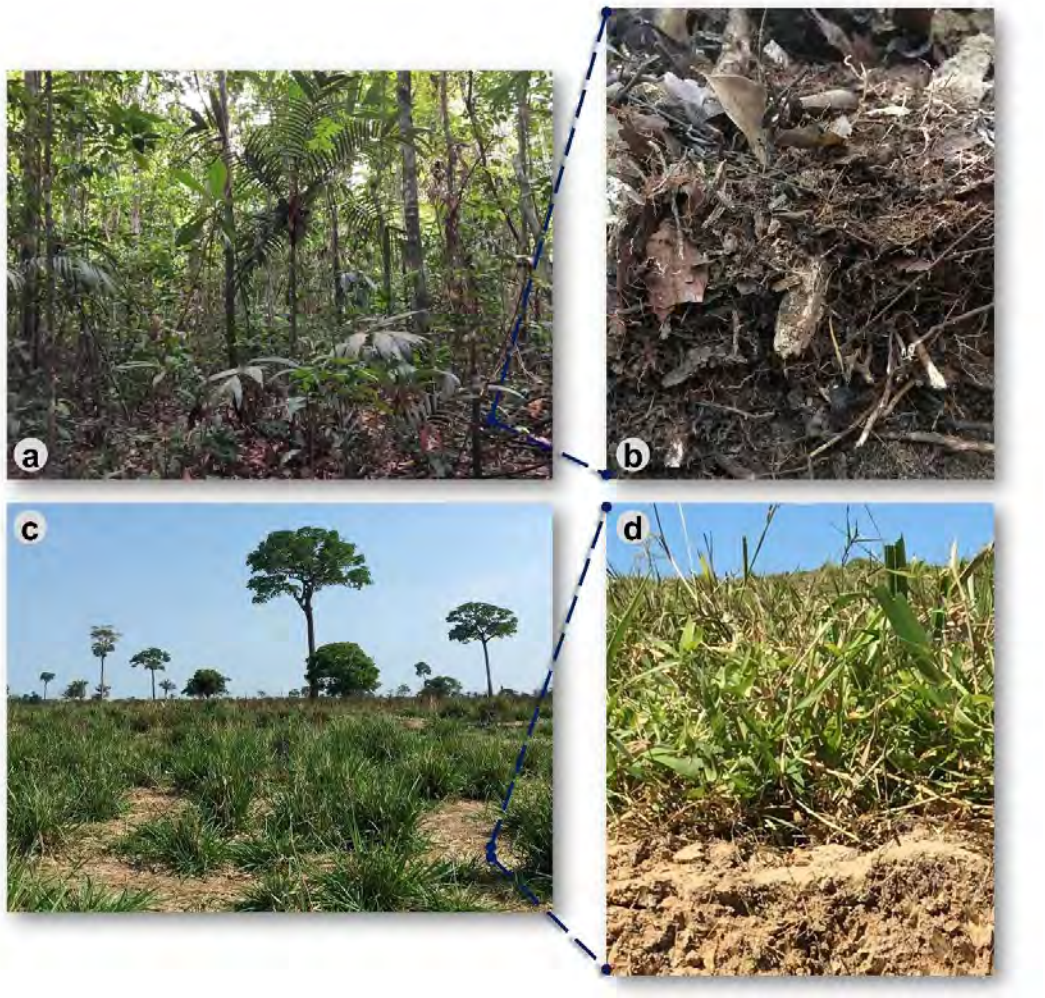

Figure 11. Illustrative representation of the evaluated land uses in the Brazilian Western Amazonia, focusing on the forest floor's decay after converting the forest to pasture. A) Rainforest where the B) forest floor (litter and the root layer on top of the mineral bulk soil) were sampled; C) pasture systems, and D) their respective soil surface with a reduced presence of organic layers.

4.4.2 Chemical and physical analysis

Soil classification was performed for all evaluated sites, using one profile per transect where pedological description and horizon soil sampling were carried out (DOS SANTOS et al., 2018; SANTOS et al., 2005; WRB, 2015). Soil physical attributes (particle size distribution and flocculation degree) were determined by the sedimentation method and reading by densimeter from the sample dispersion with 0.1 mol L^{-1} sodium hydroxide solution. The chemical analyses consisted of pH in water and KCl 1 mol L^{-1}, determined potentiometrically, in the soil: 1:2.5 solution with 1 h of contact and agitation of the suspension at the time of reading. Exchangeable sodium and potassium (Na^{+} and K^{+}) were extracted with HCl 0.5 mol L^{-1} + H_2SO_4 0.0125 mol L^{-1} ($Mehlich^{-1}$), in the proportion of 1:10 and determined by photometry of flame emission. The measurement of exchangeable calcium and magnesium (Ca^{2+} and Mg^{2+}) was performed by atomic absorption

spectroscopy and exchangeable aluminum (Al^{3+}) by titration after extraction with KCl 1 mol L^{-1} in the proportion of 1:10. The determination of potential acidity (H+Al) was carried out by titration after extraction with calcium acetate 0.5 mol L^{-1} in the proportion 1:10 and pH 7.0. The organic carbon was determined by titration of the remaining potassium dichromate with ammoniacal ferrous sulfate after the oxidation process. The calculation of derived correlations, i.e. total exchangeable bases (sum of bases = Ca^{2+} + Mg^{2+} + K^{+}), base saturation index (BS% = 100 x sum of bases / total cation exchange capacity (CEC)), and aluminum saturation index (Al saturation = (mmolc (Al^{3+}) dm^{-3} x 100) / (mmolc (effective CEC) dm^{-3}), were also analyzed (TEIXEIRA et al., 2017) at the National Soil Research Center, Brazil. The litter was properly ground and homogenized to quantify the N and C contents using CHN elemental analysis, besides the extraction of polyphenols and tannin content, following the Tropical Soil Biology and Fertility protocol (ANDERSON; INGRAM, 1993) and conducted at the National Agrobiology Research Center, Brazil.

4.4.3 DNA extraction and high-throughput sequencing

DNA extraction from the litter, root layer, and bulk soil was performed using the standard DNeasy PowerSoil kit protocol (MO BIO Laboratories Inc.), with adjustments in the time and beating intensity of the initial protocol step after adding material to the tubes containing the beads and solution C1 (FastPrep FP120-Thermo Savant BIO101; time = 40 sec; beating = 4x). Litter and the fragmented material involving root layer samples (previously sieved in a 2 mm mesh) were macerated in liquid N with pre-sterilized mortar and pestle and maintained for a minute in a water bath. Amplification of the 16S rRNA gene for DNA samples of litter, root layer, and bulk soil was performed using barcoding DNA (CAPORASO et al., 2012) with specific modifications to primer degeneracy 515F as described in Parada et al. (2016). PCR products were purified and subjected to library preparation and sequencing with Illumina MiSeq technology following the Earth Microbiome Project protocol for 16S Illumina Amplicon at the Argonne National Lab Core Sequencing Facility, USA. DNA sequence data are accessible at the MG-RAST under accession number 94905 (http://www.mg-rast.org/linkin.cgi?project=mgp94905).

4.4.4 Sequencing data processing

Sequence separation was performed in a Python environment based on primer barcodes. The 16S rRNA sequence data were further processed, aligned, and categorized using the DADA2 microbiome pipeline (https://github.com/benjjneb/dada2) by recommended parameters with quality filtering of sequence length over 250 base pairs (CALLAHAN et al., 2016). DADA2 characterizes microbial communities by identifying the unique amplicon sequence variants (ASVs) among the 16S rRNA reads. ASVs exhibit fewer false-positive taxa and reveal cryptic diversity, otherwise undetected by traditional OTU approaches (CALLAHAN; MCMURDIE; HOLMES, 2017). Further, the taxonomy was assigned for each ASV assessing the Silva taxonomic training (database v132) (QUAST et al., 2012). R packages ‘dada2’ v.1.14.0 (Callahan et al., 2016) and ‘decipher’ v.2.14 (WRIGHT; YILMAZ; NOGUERA, 2012) were used in the R 3.6.1 environment (R Team, 2018).

4.4.5 Prokaryotic metacommunity analysis and environmental variable selection

The quality step (filtering, denoising, and the removal of chimeras) on the abundance matrices was used to eliminate low prevalence sequences and sequences from Chloroplast, Eukaryota, and Mitochondria. After that, 2,735 ASVs were removed, resulting in 1,901,440 read counts, divided into 15,335 ASVs with 15,221 average counts per sample. Abundances were standardized by the median sequence depth (15,212 paired-reads). For soil variable selection, principal component analysis (PCA) was applied on the correlation matrix to obtain a smaller subset of soil variables based on their component loadings, using 'factoextra' v.1.0.7 R package (KASSAMBARA; MUNDT, 2018). Nonmetric multidimensional scaling (NMDS) was performed to visualize similarities among communities by factors (sites, land-use, and soil variables). The ecological distance was calculated with the Bray-Curtis dissimilarity matrix. Subsequently, the factors were compared through permutational analysis of variance (PERMANOVA) using Hellinger transformed data (LEGENDRE; GALLAGHER, 2001), both with 10,000 permutations. A generalized additive model with an extra penalty ($\gamma = 1.4$) was fitted to explain each selected variable's importance on the abundance matrix, with maximum likelihood as a smoothing parameter estimation method (MARRA; WOOD, 2011). The distance matrix of biotic (ASVs) and abiotic (environmental variables) data were matched using Procrustes analysis (PERES-NETO; JACKSON, 2001) to measure their correlation. We used differential heat-tree to visualize significant differences in taxonomic composition between the forest floor compartments in a pairwise Wilcoxon rank-sum test comparison using the 'metacoder' v.0.3.3 R package (FOSTER; SHARPTON; GRÜNWALD, 2017). Analyses were carried out in R environment, mainly supported by 'phyloseq' v.1.30.0 (MCMURDIE; HOLMES, 2013), 'vegan' v.2.5-6 (OKSANEN et al., 2016), and 'ampvis2' v.2.5.5 (ANDERSEN et al., 2018) packages and dependencies. Finally, linear discriminant analysis (LDA) effect size (LEfSe) (SEGATA et al., 2011) was accessed on MicrobiomeAnalyst (CHONG et al., 2020) to incorporate statistical significance with biological consistency (effect size) estimation in a non-parametric factorial Kruskal-Wallis sum-rank test to identify features with significant differential abundance. Features with at least 2.0 log-fold changes and $\alpha < 0.05$ were considered significant. All p-values were corrected by the false discovery rate method (BENJAMINI; HOCHBERG, 1995) to avoid the inflation of Type-I error due to multiple tests.

4.4.6 Diversity partitioning (α, β, and γ)

HCDT entropy has been proven as a powerful tool for measuring diversity by generalizing classical indices (MARCON; ZHANG; HÉRAULT, 2014). Here, it was turned into Hill numbers, which generate effective numbers of equally frequent species for each value of q in a unified framework, making possible the straightforward interpretation and comparison (Chao et al., 2014). The order of diversity q attaches different sensitivity to rare species, being: $q = 0$ the most sensitive (species richness); $q = 1$ all individuals are equally weighted (exponential of Shannon's entropy); and $q = 2$ is sensitive to the dominant species (inverse of Simpson index) (JOST, 2006). Because Hill numbers are continuous and have a common unit, they can be portrayed on a single graph as a function of q, leading to a "diversity profile" of effective species. Further details can be found in Chao et al. (2014). Diversity partitioning means that, in a given area, the gamma diversity of all individuals found can be divided internally, within the plot unit (alpha diversity) and between the local assembly (beta diversity) (Figure 12; DALY; BAETENS; DE BAETS, 2018) and was

calculated for all compartments of the forest floor and pasture bulk soil. Kruskal-Wallis test was used in univariate comparisons based on the global effective numbers (i.e., q 0, 1, and 2) as a single way to highlight the contribution of each compartment and all the forest floor at a given diversity scale (alpha, beta, and gamma). Analyses were performed using the 'entropart' v.1.6-1 R package (MARCON; HÉRAULT, 2015) and 'stats' v.3.6.1 (R statistical functions).

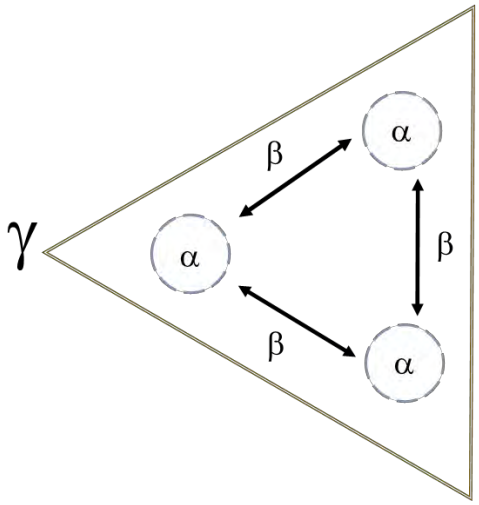

Figure 12. Diversity partitioning according to the scale of the landscape. γ-diversity is the total diversity of the landscape; α-diversity is diversity within subcommunities; and β-diversity is the diversity between the subcommunities; Adapted from Daly et al. (2018).

4.5 RESULTS

4.5.1 Gradient of soil fertility drives soil prokaryotic metacommunity structuring

A principal component analysis (PCA) on the selected soil variables (i.e., pH, BS%, Al saturation, Ca + Mg, sum of bases, and silt; Figure 13) revealed 83% and 11.2% of the explained variance on PC1 and PC2, respectively. For the extracted soil variables, no statistical differences were found between the forest and pasture of BUJ and between the pastures of BAC and MAN.

The structure of prokaryotic metacommunity (i.e., microbiota assemblies from spatially different sites) differed among the study sites (PERMANOVA, $F = 8.20$, $p < 0.001$) as well as between land uses ($F = 11.07$, $p < 0.001$) for all pairwise comparisons. Metacommunity structure was significantly correlated to the base saturation index, showing that it shifted along a gradient of soil fertility (Figure 13; $F = 9.93$, $p < 0.001$), from places with highly weathered soils (BAC and MAN forests) to those with high natural fertility (BUJ forest and pasture). We detected a significant statistical interaction between sites and land-use ($F = 3.97$, $p < 0.001$) which indicates that both factors contribute to prokaryotic community structuring, influenced by the soil type by each site and land-use characteristics, as shown further.

The Procrustes analysis identified a positive correlation between biotic and abiotic matrices (71.83%, $p < 0.001$). Generalized additive models for each extracted soil variable in the PCA revealed high deviance explained for those variables, determining its importance in mediating prokaryotic communities' distribution. Moreover, soil pH was positively associated with ASV richness.

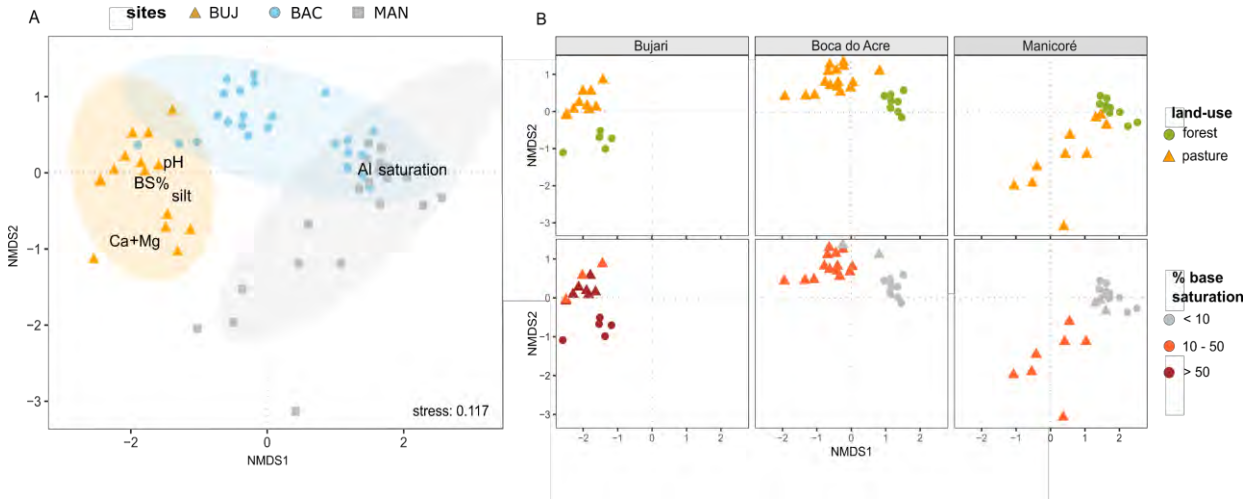

Figure 13. Land-use and soil type shape prokaryotic metacommunity structure in the bulk soils. A) Nonmetric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity among samples in the normalized ASV data of soil prokaryotic communities, highlighting the study areas and soil variables correlated with community structure; B) NMDS based on ecological distance (Bray-Curtis) of soil prokaryotic communities of each study area, highlighting the sample distribution pattern by land-use (upper boxes) and gradient of fertility (below boxes, by the base saturation index).

4.5.2 Land-use and soil type shape the predominant composition among prokaryotic soil communities

Features that most likely explain differences between land-use systems and sites were determined by linear discriminant analysis (LDA) effect size (LEfSe), and patterns were detected showing taxa associated with land-use regardless of soil type. At the phylum level, *Proteobacteria, Gemmatimonadetes, Thaumarchaeota, Rokubacteria,* and *WPS-2* were revealed as the most abundant in forest systems (Figure 14). In contrast, *Actinobacteria, Chloroflexi, Firmicutes,* and *Bacteroidetes* were the phyla with the highest differential abundance in pasture systems. For BAC, we found eight significantly more abundant phyla in pasture soils and four in the forest. Both BUJ and MAN had the same number of predominant phyla among their land uses. When comparing the same land-use among different sites, we observed that the BUJ forest hosts the largest significant number of predominant phyla compared to other sites. *Verrucomicrobia,* in BUJ, and *Acidobacteria,* in BAC, are the most prevalent phyla in pasture and forest soils, respectively (Figure 15).

4.5.3 The structure and composition of prokaryotic metacommunity in the forest floor reflect land-use as a biotic selector

Prokaryotic metacommunity structure differed significantly among the litter, root layer, and bulk soils, and this result was consistent among all studied sites (Figure 14; PERMANOVA, $F = 18.08$, $p < 0.001$). The prokaryotic metacommunity structure of the litter communities contrasted with those found in other compartments of the forest floor (Figure 16). Differences in the prokaryotic metacommunity among sites were associated with variations in litter chemical composition (Procrustes analysis: 63.2%, $p < 0.001$), mainly due to the polyphenol content, N content, and C:N ratio (Figure 16). All forest floor compartments were compared among themselves and with the pasture bulk soil. Taxa that were enriched or reduced were identified (Figure 15 and 16). *Chloroflexi, Proteobacteria, Firmicutes,* and *Verrucomicrobia* were the most statistically different (LDA; $p < 0.001$). *Proteobacteria* was the only phylum present in all forest compartments, especially in the litter (> 60% relative abundance; $p < 0.001$, LDA = 3.6). These patterns were found to be similar in all sites. *Planctomycetes* were the most representative group in the root layer of the forest ($p < 0.001$, LDA = 2.05) despite their low relative abundance (Figure 14C). Overall, 30.2% of ASVs are shared among the forest floor's compartments; 22.6% between BAC and MAN; 13.1% between BAC and BUJ, and only 1.3% between BUJ and MAN. BUJ has 1491 (14.2%) restrict ASVs in its microbial communities (Figure), reflecting the distinct chemical composition in the forest floor's compartments in relation to the other sites.

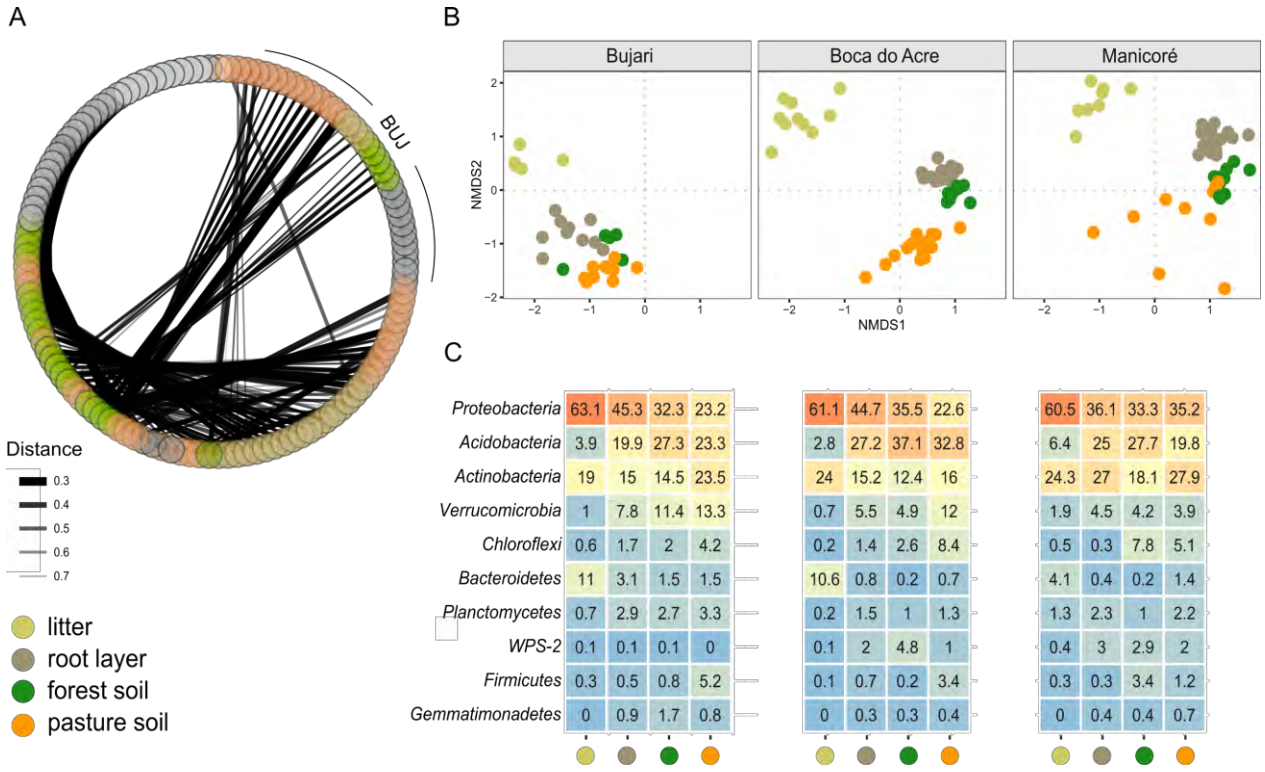

Figure 14. Co-occurrence of prokaryotic metacommunity between sites based on forest edaphic environment compartments and pasture bulk soil. A) Co-occurrence based on compartments (litter, rhizosphere, forest and pasture bulk soil) and study areas; the thickness of the links is proportional to the strength of the interactions; B) NMDS by compartments and sites (BUJ, BAC and SAM); distance measured by Bray-Curtis based on the abundance of ASVs from each sample point; C) Relative abundance of Bacteria (phylum level) in compartments of edaphic environments and pasture soil.

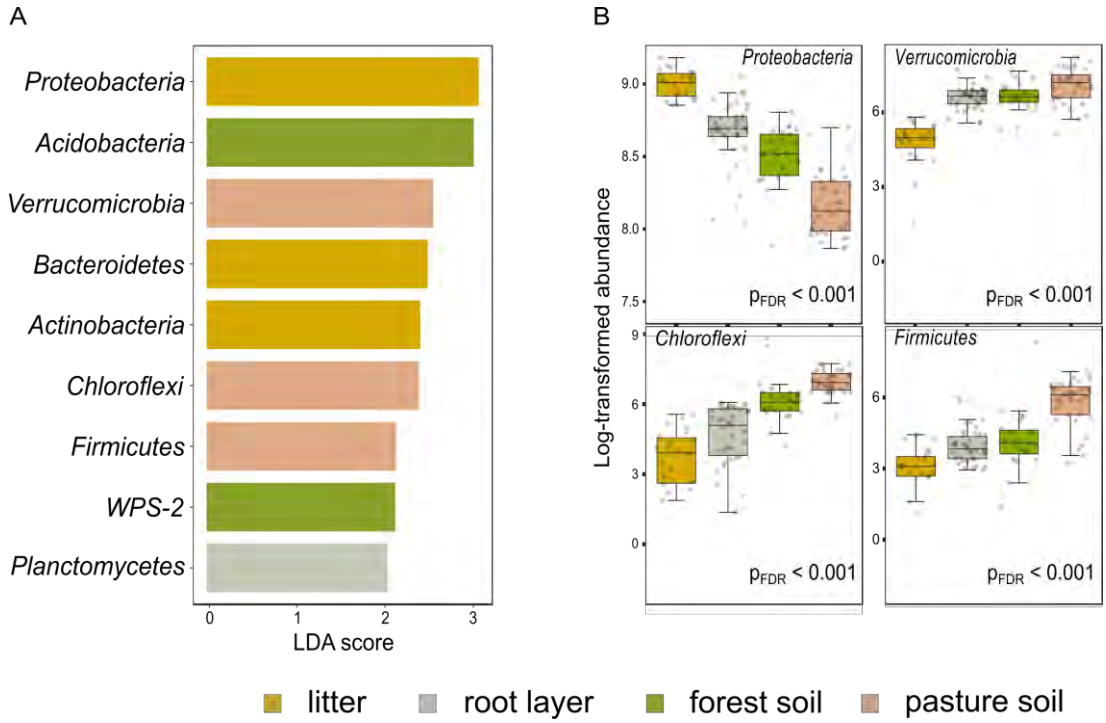

Figure 15. Differential abundance among the most relevant taxa in the forest edaphic environment and pasture bulk soil in the Western Brazilian Amazon. LEfSe multivariate analysis to significant differential abundances (false discovery rate adjusted p-value (pFDR) < 0.001) with LDA > 2.0; A) Features selected between the compartments of the forest edaphic environment and the pasture bulk soil; B) First four features based on pFDR < 0.001, without the application of the LDA.

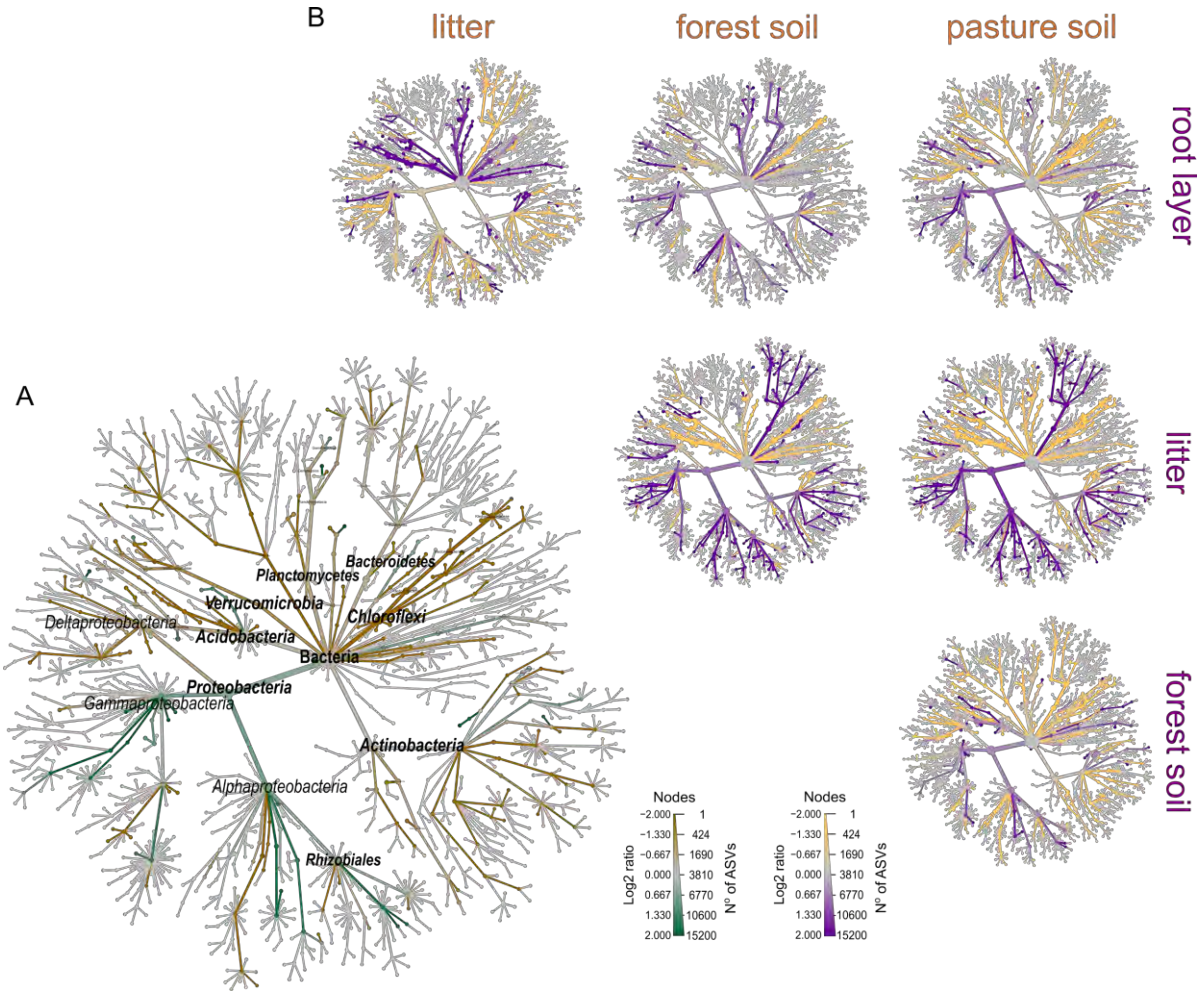

Figure 16. Phylogenetic differential heat tree highlighting the most expressive features among the compartments of the edaphic environment. A) Predominance of phylogenetic groups in forest edaphic environment (green color) and pasture bulk soil (brown color); B) Pairwise comparison between each compartment; The color of each branch represents the log-10 ratio of median proportions of reads observed at each compartment. Only significant differences are colored, determined using a Wilcoxon rank-sum test followed by a Benjamini-Hochberg (FDR) correction for multiple comparisons.

4.5.4 Forest floor reveals prokaryotic diversity and spatial turnover in Brazilian Western Amazonia

Diversity partitioning analysis showed that the ASV richness (q = 0) in bulk soils is significantly higher in pastures than forests for all diversity scales and study sites, especially for MAN (Figure 17). Beta ($\chi^2 = 6.94$, $p < 0.001$), and gamma diversity ($\chi^2 = 5.43$, $p = 0.013$) was also significantly higher in pasture bulk soil, except for BUJ ($p > 0.05$). The effective number of dominant ASVs was similar (q = 2) for any diversity scale, as well as in the comparison between forests and pastures, meaning that both systems have a similar number of dominant groups in the bulk soil. Nevertheless, when the forest floor was taken as a whole, that is, when the metacommunities in the litter, root layer, and bulk soil were analyzed together, we observed that

the differences in the alpha "local" diversity between forest and pastures were no longer observed, as previously found in the comparison between bulk soils. Only BAC showed a statistically higher effective number of species in its pastures for all orders of diversity q. BUJ had the highest alpha diversity for both litter, root layer, and bulk soils compared to the other study sites. Especially, the ASV richness (q = 0), as well as Shannon diversity (q = 1) and Simpson dominance (q = 2) of the forest floor showed the highest beta diversity for all study sites, which indicate a more prominent spatial turnover of the prokaryotic community. For the gamma diversity, only the forest floor of BUJ had a significant global difference in the effective number of species between forest and pasture ($\chi^2 = 6.64$, $p = 0.009$), although the similar higher ASV richness (q = 0) in the forest floor than in the pasture bulk soil for all study regions.

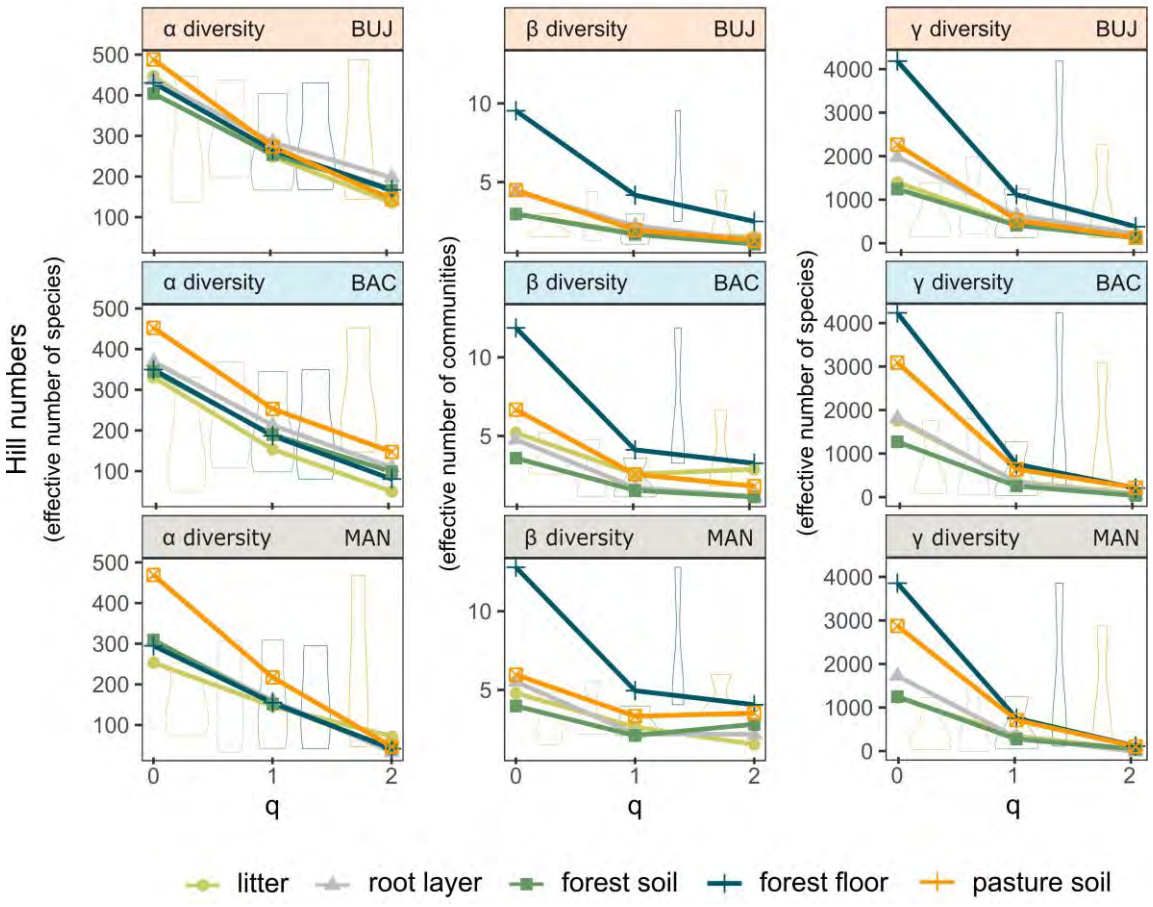

Figure 17. Diversity partitioning analysis evidencing the heterogeneity of PM across land uses. Alpha (α), beta (β) and gamma (γ) (i.e., local, community, and regional) diversities for the forest and pasture bulk soils, the forest litter and rhizosphere, and the forest edaphic environment in each site. Hill numbers (q = 0, ASV richness), (q = 1, exponential of Shannon's entropy for equally weighted ASVs) and (q = 2, inverse of Simpson index for dominant taxa).

4.6 DISCUSSION

4.6.1 Prokaryotic metacommunity reflects the synergistic interaction between land-use and soil type

Multiple analyses based on the next-generation sequencing approach allowed us to support our first hypothesis that soil type, rather than land-use patterns, mainly leads to structuring the prokaryotic metacommunity in bulk soils. This finding highlights that soil variables, especially those related to soil fertility, such as pH and base saturation, are the major attributes driving the prokaryotic community structuring in bulk soils.

Our argument is based on the observation that communities from the most distant geographic areas (± 650 km; BAC to MAN) showed more remarkable structural and compositional similarities (Bray-Curtis distance = 0.51) than communities from nearby sites (± 150 km; BUJ to BAC; Bray-Curtis = 0.87). This distinction reflects the influence of different soil-forming processes on microbial community structuring. Soils of the state of Acre mostly come from weathering sedimentary rocks, and specifically, those found in this study are a patch of naturally eutrophic soil, such as Luvisols (BERNINI et al., 2013). Predominantly, BAC and MAN have Acrisols and Ferralsols, highly weathered soils, covering most of the Amazon basin (SCHAEFER et al., 2017), and developed on sandstones and claystones, and mainly formed on remnants of ferrallitic plateaus and convex hills which are not flooded (SOUZA et al., 2018). The gradient of soil fertility across soils with distinct pedogenesis and weathering degrees is a major contribution of this study to understanding how microbiota are modeled under the same land-use system. Soil pH may not directly alter prokaryotic community structure but may be considered an integrating variable that provides an index of soil conditions (LAUBER et al., 2009). Many soil attributes, such as nutrient availability, cationic metal solubility, organic C characteristics, soil moisture condition, and salinity, are often directly or indirectly related to soil pH (BISSETT et al., 2011; SULEIMAN et al., 2013). However, recent studies indicate that bacterial community assembly processes differ concerning soil pH, with near-neutral pH leading to more stochastic communities, whereas extreme conditions lead to more deterministic assembly and clustered communities (TRIPATHI et al., 2018). Thus, the influence of variables such as temperature is mainly revealed where soil pH is relatively constant (NOTTINGHAM et al., 2018).

Our results consistently support a cause-effect relationship between soil pH and alterations in the natural structure and composition of the soil microbiota due to the land-use conversion (BERKELMANN et al., 2018; GOSS-SOUZA et al., 2017; JESUS et al., 2009; MENDES et al., 2015; NAVARRETE et al., 2015). Moreover, regarding the taxonomic approach of communities, we observe a clear community fingerprint throughout land uses, even considering the different soil types. *Actinobacteria* were dominant in the pastures to the detriment of *Proteobacteria*, which were considerably abundant in the forest floor, especially in the litter. Increases in the relative abundance of *Actinobacteria* and *Chloroflexi* populations were highlighted in Fierer et al. (2012) and Mendes et al. (2015). *Actinobacteria* are functionally related to organic substrate decomposers and produce spores, allowing this group to maintain its activity in more anthropized systems (VENTURA et al., 2007). Some groups of the *Chloroflexi* are thermophilic aerobes, having the ability to develop their metabolism at high temperatures, also keeping an important relationship in the decomposition of organic matter (YAMADA et al., 2005) and, consequently, predominance in pasture soils. In turn, *Proteobacteria* are usually related to high levels of organic C and have been extensively reported as a land-use change indicator as its high abundance is drastically reduced after the conversion of

the rainforest into pastures (DE CARVALHO et al., 2016; MENDES et al., 2015; NAVARRETE et al., 2015). *Proteobacteria*, specifically *Alphaproteobacteria,* and *Gammaproteobacteria*, which were highly evident in our study, mainly in the litter layer (see Fig. 5), are functionally important in natural systems known to undergo weak soil perturbation and provide copiotroph habitats rich in recalcitrant organic matter (PASCAULT et al., 2013). They are also closely related to methane oxidation (CH_4) due to their methanotrophic characteristics, helping to mitigate these gas' emissions by controlling the production-consumption balance within systems with lower anthropic disturbance, such as forests (TATE, 2015).

4.6.2 Role of the prokaryotic metacommunity in the forest floor and deforestation as a risk for its maintenance

The tropical forest floor undoubtedly plays a vital role in the biodiversity and ecosystem functioning on a global scale (POORTER et al., 2015). The biogeochemical cycles in that ecosystem regulate the most extensive terrestrial C storage, maintaining high biomass and productivity, although mainly growing on nutrient-poor soils (FINZI et al., 2011; SAYER et al., 2020). However, the rapid advancement of livestock expansion represents a high risk for its maintenance because the forest floor is irreversibly affected during the forest-to-pasture conversion, with no subsequent replacement of some of its compartments. Some efforts to evidence nutrient retention and uptake in the forest floor have been made (SAYER et al., 2020; SAYER; TANNER, 2010), considering that the mineral soil measurements only represent a small part of the picture. Hence, a better understanding of the role of the forest floor's prokaryotic communities and how they are impacted by deforestation is essential to predict consequences in the face of global changes (LLADÓ; LÓPEZ-MONDÉJAR; BALDRIAN, 2017; RILLIG et al., 2019).

Firstly, our investigation of forests in the Western Amazonia suggests that litter prokaryotes apparently do not have an intrinsic relationship with the root layer and soil microbiota; therefore, they are not directly influenced by soil attributes. It is noteworthy that litter microbiota are likely predominantly endophytic and related to the forests' floristic composition and phenology patterns (BUSCARDO et al., 2018). A specific litter quality chemically related to the forest phytophysiognomy is added to the forest floor, providing different drivers for microbial community structuring (BUSCARDO et al., 2018; RITTER et al., 2018, 2020). Nonetheless, plant diversity and community composition are influenced by geology and physicochemical soil properties (HIGGINS et al., 2011; RITTER et al., 2018), which is indirectly important to explain variations in composition and structure of the litter microbiota. *Proteobacteria, Actinobacteria,* and *Bacteroidetes* were the most abundant phyla in that compartment, as already evidenced by Purahong et al. (2016) and Tláskal et al. (2016). Moreover, we observed differences in the communities between study sites, explained by the litter's chemical composition. We detected a higher content of polyphenols, tannins, and C:N ratio, mainly in the litter of the MAN's forests, which may be related to the highest relative abundance of *Actinobacteria* (LEWIN et al., 2016) and a smaller abundance of *Bacteroidetes* (XUE et al., 2016) compared to the other study sites.

The fine-root production and turnover have a significant plant detritus input to the soil. It is also a key energy source to soil microbiota, and consequently, a major pathway of nutrient flux in terrestrial ecosystems (YUAN; CHEN, 2010; ZECHMEISTER-BOLTENSTERN et al., 2015). In our study, the root layer-associated communities also showed significant structural differences among the study sites but sharing similarities with the bulk soil due to its transient position on the forest floor. Despite the differences in community structure, we observed a clear enrichment of

Planctomycetes in the root layer for all study sites. Some planctomycetes may be involved in degrading polymeric organic matter (IVANOVA et al., 2018). However, experimental data remain scarce due to the low number of characterized representatives of this phylum. The higher relative abundance of *Planctomycetes* in the root layer has already been reported for the Amazonian rainforest (FONSECA et al., 2018). Nevertheless, more studies are needed to understand the ecological role of planctomycetes in the root layer of tropical rainforests and its potential representativity for that ecological niche.

4.6.3 Forest floor as an ecosystem for accessing microbial diversity in tropical forests

Although our results agreed with previous studies that have identified higher alpha diversity in pasture soils compared to forests, a better understanding of microbial turnover and gamma diversity is still on demand, as pointed out by Petersen et al. (2019) in a recent meta-analysis that tackled the soil microbiota in tropical land uses. Our diversity partitioning analysis does not indirectly indicate a positive correlation between plant and soil prokaryotic beta diversity, as found by Prober et al. (2015), neither does it indicate the reduction of spatial heterogeneity in pastures introduced after deforestation, as evidenced by Rodrigues et al. (2013) in the Western Amazonia and Goss-Souza et al. (2017) in the Atlantic Rainforest. Our results agreed with the findings described by de Carvalho et al. (2016), who found a higher beta diversity for soil prokaryotes in more altered land uses of the Eastern Amazonia, such as pastures, especially for ASV richness ($q = 0$) and Shannon diversity ($q = 1$).

Nevertheless, when forest litter and root layer were taken into account with the bulk soil, we detected a higher effective number of communities (beta diversity) within all studied forests rather than pastures. Since similar trends were found among the study regions, geographically distant and dissimilar in the composition of the measured soil and litter variables, the forest floor's biodiversity might confer similar ecological functioning abilities to the forest ecosystem, such as nutrient cycling and C sequestration, leading to a positive diversity–stability relationship at the landscape scale. Moreover, aspects related to the forest floor's functional redundancy are crucial for further investigation. BUJ was the only site where the global (q 0, 1, and 2) gamma diversity significantly differed between the forest floor and pasture bulk soil. The non-overlapping of the gamma diversity in less sensitive q values ($q = 1$ and $q = 2$; see Fig. 6) may indicate that the higher natural fertility found in BUJ soils, in addition to the higher labile N content in its forest litter, should support a more stable prokaryotic diversity than the other study regions that only showed a significant effective number of species in the most sensitive Hill number (i.e., ASV richness, $q = 0$). Our results partially corroborate our second hypothesis since we have not seen consistent increases in alpha "local" and gamma "regional" diversities after including all forest floor compartments in the diversity partition analysis. Intriguingly, when observed individually, the litter, root layer, and bulk soil compartments do not give clear information about the turnover of prokaryotic communities, so an integrated interpretation of this system is necessary. Similar findings were reported by Ritter et al. (2018), where the correlation between OTU diversity in litter and soil was weak for prokaryotes and non-significant for eukaryotes. Considering the fungal communities, which play a pivotal role in tropical biodiversity (RITTER et al., 2020), Berkelmann et al. (2020) reported a decrease in the diversity of functional genes but an increase in taxonomic diversity, comparing a gradient from rainforest to agriculturally managed systems in Sumatra (Indonesia), indicating prevalence in less versatile species in monoculture soils or more functionally redundant taxa. Since the habitat type strongly shape the fungal community

composition (RITTER et al., 2018, 2020), broad efforts should be made to measure a wider portion of soil biodiversity, aiming for a better understanding of the effects of land-use intensification on complex edaphic microbiota to predict risks to the ecosystem functioning, which are essential for the maintenance of life.

4.7 CONCLUSION

Altogether, our results support previous studies that show a strong relationship between soil pH and fertility on the structure of prokaryotic metacommunity in the Amazon region. This relationship was observed at the local level, as a consequence of forest-to-pasture conversion, and at the regional level, due to natural differences in soil fertility. All pasture bulk soils have prokaryotes more correlated with increases in soil pH and base saturation, resulting in higher alpha, beta, and gamma diversities. Beta and gamma diversities were generally higher in the forests when the forest floor was considered a whole, highlighting increases in microbial heterogeneity across space; however, at the plot-scale (alpha diversity), it remained higher in pasture bulk soils. By adding the forest litter and root layer to the bulk soil in our measurements, we demonstrate that prokaryotes vary in their community structure and composition among the forest floor compartments, with a relevant site-specific influence. Our findings shed light on the importance of including the forest floor compartments to understand the dynamics of microbial communities across tropical ecosystems, besides giving new perspectives on the issue of biotic homogenization. Other pasture floor compartments should be characterized and included to generate a better picture of the presented scenario for future efforts.

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5. CHAPTER III

LINKING SOIL TO MOUTH: UNRAVELING THE CASCADE-EFFECT OF LAND-USE CHANGE AND CATTLE PERIODONTITIS

5.1 RESUMO

A patogênese da periodontite em ruminantes, que se manifesta pela destruição dos tecidos periodontais causando severa perda de peso em casos mais crônicos, é considerada uma doença multifatorial causada por complexos de microrganismos e tem sido historicamente invocada em decorrência do desequilíbrio ecológico em sistemas naturais. No entanto, os processos ecológicos que desencadeiam as infecções ainda não foram totalmente elucidados. Para atacar esta questão, este estudo avaliou sistemas de pecuária extensiva em um gradiente inter-regional de fertilidade do solo na Amazônia Ocidental brasileira, com casos recentemente relatados de doença periodontal em bovinos. Em geral, as pastagens foram abertas de forma semelhante após o corte e queima das florestas primárias para a pecuária extensiva. Após o emprego de uma abordagem multidisciplinar de análises, os resultados aqui obtidos revelam que o continuum solo-planta-animal pertencente aos sistemas com baixo nível de severidade da doença (BNS) possuem predominância das classes *Bacilli* e *Gammaproteobacteria*, maiores teores de cobre no solo, bem como maior conteúdo de macro e microminerais na forragem, sugerindo um ambiente mais supressivo nessas pastagens. Pastagens com alto nível de severidade da doença (ANS) apresentaram maior abundância de *Bacterodia* nas forrageiras e animais, e *Actinobacteria* nos solos, maior relação C:N do solo, o que indica uma menor capacidade nutricional geral do sistema. Ainda, foi observada uma clara diferença entre os sistemas ANS e BNS em relação à estrutura das comunidades núcleo (*core*) do solo, forragem e gado, sugerindo que os sistemas ANS são mais suscetíveis às mudanças no ambiente edáfico. Foi encontrada uma maior diversidade alfa e gama em todos os componentes do continuum solo-planta-animal do sistema ANS, o que sugere um maior estresse ambiental sobre a comunidade procariótica associada. A rede de coocorrência global, que integra a microbiota do solo, forragem e gado, evidenciou que o continuum pertencente aos sistemas BNS foi estruturado em poucos, mas altamente estáveis e conectados módulos, ao contrário dos sistemas ANS, que foi estruturado por um maior número de módulos menores e interconectados. Além disso, a análise de qPCR evidenciou que os sistemas suscetíveis a doenças são mais propensos a aumentos na abundância do gene da biossíntese de estreptomicina no solo, forragem e gado, sendo este relatado previamente por facilitar a aderência de bactérias patogênicas às células epiteliais bovinas. No geral, a abordagem multidisciplinar deste estudo permitiu avançar na compreensão sobre a complexidade associada ao efeito-cascata da conversão de floresta em pastagem e as possíveis consequências na biodiversidade do solo e na saúde do gado.

Palavras-chave: Periodontite bovina. Uso antropogênico da terra. Ambiente supressivo. Gene *strB1*.

5.2 ABSTRACT

The pathogenesis of periodontitis in ruminants, which manifests itself by the destruction of periodontal tissues causing severe weight loss in more chronic cases, is considered a multifactorial disease caused by complexes of microorganisms and has been historically invoked as a consequence of the disruption of the ecological balance in natural systems. Nonetheless, the ecological process that trigger the infections have not yet been fully elucidated. To tackle the issue, this study assessed extensive livestock systems across an interregional gradient of soil fertility in the Brazilian Western Amazonia, with recently reported cases of the periodontal disease in cattle. In general, the pastures were similarly opened after slash-and-burning of the pristine forests to employ extensive cattle ranching. After employing a set of multivariate analysis, the results obtained here reveals that the soil-plant-animal continuum belonged to pastures with low severity level of disease (LSL) have a predominance of the classes *Bacilli* and *Gammaproteobacteria*, higher levels of soil copper, and macro and microminerals in the forage, suggesting a more suppressive environment in those pastures. Pastures with high severity level of the disease (HSL) showed higher abundance of *Bacterodia* in forage and animals, and *Actinobacteria* in soils, higher soil C:N ratio, which indicates an overall lower nutritional capacity of the system. Yet, it was observed a clear difference between HSL and LSL systems regarding the core community structure of the soil, forage, and cattle, suggesting that HSL systems are more susceptible to the changes in the edaphic environment. It was found a higher alpha and gamma diversity in all the components of the soil-plant-animal continuum of the HSL system, which suggests greater environmental stress upon the associated prokaryotic community. The global co-occurrence network, that integrates the soil, forage, and cattle microbiota, evidenced that the continuum belonged to LSL systems was structured in few, but highly stable and connected modules, unlike HSL systems, which was structured by a greater number of smaller and interconnected modules. Moreover, the qPCR analysis evidenced that the disease-susceptible systems was more prone to increases in the abundance of streptomycin biosynthesis gene in soil, forage, and cattle, which was previously reported to facilitate adherence of pathogenic bacteria to bovine epithelial cells. Overall, the multidisciplinary approach used in this study increased the understanding of the complexity associated with the cascade-effect of forest-to-pasture conversion and consequences on soil biodiversity and cattle health.

Keywords: Cattle periodontitis. Anthropogenic land-use. Suppressive environment. *strB1* gene.

5.3 INTRODUCTION

Delineating the cascade-effect of biotic and abiotic disturbances triggered by landscape simplification is a challenge still timidly faced by the current applied research, as is the case of the consequences manifested as pathologies for humans and animals (GOTTDENKER et al., 2014). However, this issue is undoubtedly relevant given the advance of anthropogenic pressure on the sources of biodiversity, with particular attention to tropical rainforests (NEWBOLD et al., 2014). The forest-to-pasture conversion promotes drastic effects upon the edaphic environment, including the decline of microbial biodiversity residing in the forest floor (ROCHA et al., 2021), changes in soil nutrient balance (BRAZ; FERNANDES; ALLEONI, 2013), and physical-water dynamics (NOBRE et al., 2016), which leads to the structuring of more adapted microbiomes to disturbed land uses.

Since the 1960s, the pioneering research by Dr. Jürgen Döbereiner[1] and his team has provided observational, quantitative, and qualitative evidence that consistently indicates that periodontal disease in ruminants is a multifactorial infectious disease caused by complexes of microorganisms. An aggressive manifestation of this disease has been called "swollen face" ("cara-inchada dos bovinos - CIb" *in Portuguese*) in Brazil (DÖBEREINER et al., 2000) and internationally known as "broken mouth disease", which affected herds in New Zealand, the UK, and Scotland (BORSANELLI et al., 2016; MCCOURTIE et al., 1990; RIGGIO; JONSSON; BENNETT, 2013). Symptomatically, the disease manifests itself by the destruction of periodontal tissues and loosening and shedding of cheek teeth, which turns chewing difficult, causing severe weight loss in more chronic cases (DÖBEREINER et al., 2000). Reported as a microbiological response to changes in the soil environment, the onset of the periodontal disease is, particularly, a suspected consequence of the forest-to-pasture conversion or pasture renewal as well. It has been hypothesized that both within-farm (e.g., type and pasture quality) and broad scale aspects (e.g., environmental conditions and geology) can lead to differences in the occurrence and severity of the disease among farms. Although the causal drivers are not yet fully described (HOLMES; THOMAS; HAMEROW, 2021), previous reports suggest that sub-inhibitory soil streptomycin doses facilitate the biofilm formation, as well as the adherence of pathogenic bacteria to bovine epithelial cells (GRASSMANN et al., 1997; KOPP et al., 1996; KUMAR; TING, 2016). Moreover, reports suggest that good balance in macronutrients of the forage and copper promote better quality of herd health, decreasing infection in previously diseased cattle (MORAES; SILVA; DÖBEREINER, 1994). Recent studies have shown that the dimension of abiotic soil transformations is an interaction between the impact of the clearance of the pristine vegetation cover and the soil genetic factors (see main findings of chapter I). This matrix between genetic and anthropogenic factors apparently confers different levels of susceptibility of the pasture edaphic environments to environmental changes, defining the magnitude of the effects upon soil attributes. This observation especially refers to the soil chemical variables, which are highly altered by water dynamics (e.g., iron oxidoreduction), root density, plant morphology and metabolism (DAVIDSON et al., 2000), intensity of solar radiation, as well as the influence of grazing and input of animal excreta (MÜLLER et al., 2004).

Land-use change is a commonality between converted pastures, thus, a complete comprehension about the set of drivers that might turn some systems more prone to triggering outbreaks of bovine periodontitis than others is still a gap of knowledge. Therefore, applying a multidisciplinary analysis approach to elucidate possible correlations between edaphic environment disturbances and the cattle periodontal disease becomes necessary. To tackle this issue, we assessed extensive livestock systems across an interregional gradient of soil fertility in the Brazilian Western Amazonia, with recently reported cases of the periodontal disease in cattle. Overall, the pastures were similarly opened after slash-and-burning of the pristine forests to employ extensive cattle ranching. Within this panorama, merging genomic tools to a suite of measurements of abiotic variables of soil and forage components, we aimed at deciphering some relevant

[1] DUTRA, I. S.; COLLING, A.; DRIEMEIER D.; BRITO, M. F.; UBIALI, D. G.; SCHILD A. L.; RIET-CORREA, F.; BARROS C. S. L. L. Jürgen Döbereiner: *a life dedicated to science*. **Pesquisa Veterinária Brasileira**, v. 39, p. 1-11, 2019.

attributes among the soil-plant-animal continuum that consistently indicate differences in the general signatures between disease-susceptible and healthy systems. In this study, it was hypothesized that the high severity level system of bovine periodontitis promotes greater conditions for the disruption of the microbiota of the soil-plant-animal continuum in response to local environmental characteristics. The specific hypothesis is that the greater disturbance of high-severity systems leads to greater streptomycin biosynthesis in soil, forage, and consequently in the oral environment of cattle, increasing the conditions for the development of infections. Specifically, we sought to (i) identify representative abiotic variables that differentiate systems with high and low severity level (HSL and LSL) of the disease; (ii) define biomarker taxa in the core microbiota of the soil, forage, and cattle subgingival biofilm between HSL and LSL systems; (iii) measure the co-occurrence networks parameters of the soil-plant-animal continuum microbiota; and (iv) predict functional metabolic profiles of the prokaryotic metacommunity, and quantify the streptomycin biosynthesis gene (*strB1*) in DNA samples from soil, forage, and cattle subgingival biofilm.

5.4 MATERIAL AND METHODS

5.4.1 Study area selection and sampling

This study took place in three different regions of the Brazilian Western Amazonia. Localities highly affected by the intense advance of deforestation rates in recent years were surveyed based on reports and consultations with regional public institutions. Five farms of extensive livestock production were selected in the municipalities of Bujari (state of Acre), Boca do Acre (state of Amazonas), and Manicoré (state of Amazonas) (Table 1). Those farms attended the following *a priori* criteria: 1) Conserved forests adjacent to pasture areas; 2) No management history of liming and chemical fertilization; and 3) Availability of adequate structure for clinical animal examination and subgingival biofilm sampling.

Sampling was performed in August 2017, following the procedures found in Rocha et al. (2021) among eight pasture areas. We used linear transects with 200 m in length, including five sampling points 50 m apart from each other and varying according to the sampling area. Bulk soil (0-10 cm) and forage leaf (at the grazing height of the animals) samples were collected for both characterization of abiotic variables and molecular analysis. A composite sample was formed from three pooled subsamples at each sampling point (Figure 18). All collected material for molecular analysis was immediately packed in sterile pouches, kept on ice, and refrigerated at -80ºC in the shortest time possible.

a) Criteria for cattle selection and examination

Only adult beef cattle in herds of ≥ 200 animals with at least one year of grazing on extensive pasture (i.e., feeding exclusively on pasture, except for mineral salt without additives, and placed *ad libitum*), were included in the research. The selection of herds for clinical examination took place with the owners, considering the *a priori* established conditions.

After the interpretation of the clinical examinations, depending on the prevalence of adult animals with periodontal lesions, the farms were divided into areas of high or low severity level of periodontitis (HSL and LSL, respectively). Farms were classified as HSL when the prevalence of animals with periodontal lesions was higher than 20% of the herd. On the other hand, those areas with an occurrence of less than 5% of periodontal lesions in the evaluated animals were classified as LSL (Table 1).

5.4.2 Soil chemical and physical analysis

Information on physical and chemical soil characterization can be found in section 3.4.2.

Table 1. General information on properties classified as high and low severity level of cattle periodontitis in the Brazilian Western Amazonia.

Sites	General information	Severity level
Bujari, AC[1] 9°49′22″S 67°56′51″W 196 m.a.s.l.	Farm 1: 5,900 ha of total area being 1,900 ha of pastures varying in conservation conditions; Herd of 5,000 cattle. Pasture A: Newly formed pasture (< 7-year-old), predominance of Urochloa brizantha cv. MG5 and U. humidicola; Stagnic Luvisol (Luvissolo Háplico Pálico gleissólico, loamy/clayey texture, moderate A horizon) as predominant soil type. Pasture B: 25-year-old pasture, U. decumbens predominantly; Stagnic Plinthosol (Plintossolo Argilúvico Alumínico gleissólico, loamy/clayey/very fine clayey texture, moderate A horizon).	Low (LSL)
Boca do Acre, AM 2 8°44′26″S 67°23′3″W 99 m.a.s.l.	Farm 2: 3,000 ha of total area, 1,000 ha of pastures; Herd of 1,000 cattle. Pasture C: Newly formed pasture, predominance of Panicum maximum cv. Mombaça and U. humidicula; Xanthic Ferralsol (Latossolo Amarelo Alumínico típico, clayey/very fine clayey texture, moderate A horizon) as predominant soil type. Pasture D: 30-year-old pasture, U. humidicola and U. brizantha cv. Marandu predominantly; Xanthic Ferralsol (Latossolo Amarelo Distrófico argissólico, loamy texture, moderate A horizon).	Low (LSL)
	Farm 3: 8,000 ha of total area, 5,000 ha with pastures; Herd of 6,000 cattle. Pasture E: Newly formed pasture, predominance of U. brizantha cv. Xaraés and MG5; Pisoplinthic Ferralsol (Latossolo Amarelo Distrófico petroplíntico plintossólico, loamy/gravelly clayey texture, moderate A horizon) as predominant soil type. Pasture F: 20-year-old pasture, U. brizantha cv. Marandu and U. humidicola. Plinthic Ferralsol (Latossolo Amarelo Distrófico plintossólico, clayey texture, moderate A horizon).	High (HSL)
Manicoré, AM 5°48′34″S 61°18′2″W 32 m.a.s.l.	Farm 4: 1,350 ha of total area, 400 ha with pastures; Herd of 300 cattle. Pasture G: Newly formed pasture, predominance of P. maximum cv. Mombaça; Xanthic Ferralsol (Latossolo Amarelo Distrófico argissólico, loamy texture, moderate A horizon) as predominant soil type.	High (HSL)
	Farm 5: 1,500 ha of total area, 550 ha with pastures; Herd of 800 cattle. Pasture H: Newly formed pasture, predominance of U. brizantha cv. MG5; Xanthic Ferralsol (Latossolo Amarelo Distrófico argissólico, loamy/clayey texture, moderate A horizon) as predominant soil type.	High (HSL)

[1] State of Acre; 2 State of Amazonas; * m.a.s.l. – meters above sea level; Soil classification followed the World Soil Reference (WRB, 2015) and the Brazilian System of Soil Classification (SANTOS et al., 2018).

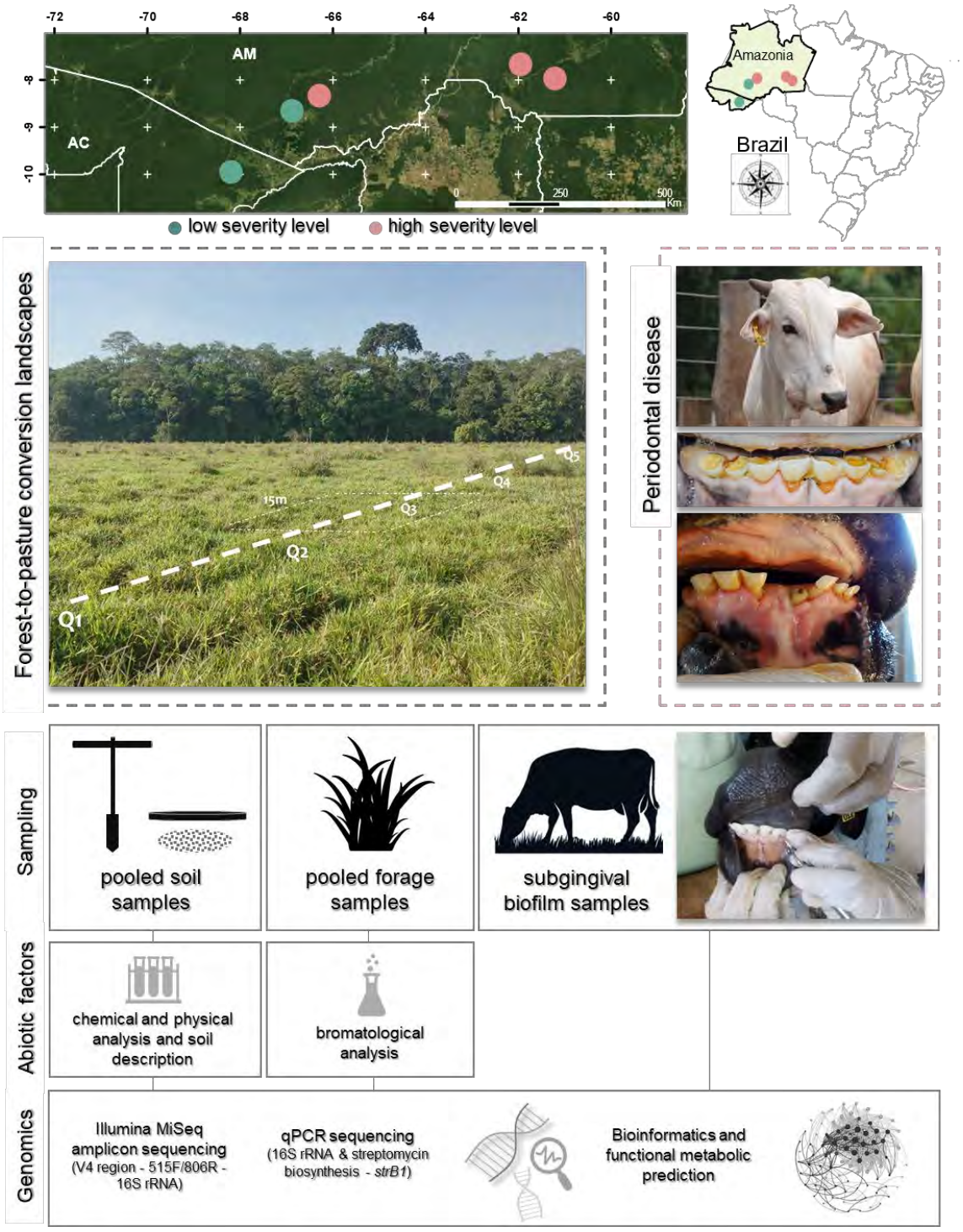

Figure 18. Illustrative methodological scheme, highlighting the study regions in the Western Brazilian Amazonia, aspects of periodontal disease, the sampling design of soils, forage, and cattle subgingival biofilm for subsequent abiotic characterization, DNA extraction, and genomic analyses.

5.4.3 Bromatological analysis

The determination of the total-N content occurred by sulfuric digestion followed by distillation by the micro-Kjeldahl method following the description of AOAC (1995). The plant crude protein content was calculated by multiplying the total-N content by 6.25 (SILVA; QUEIROZ, 2002). The concentration of macronutrients (N, P, K, Ca, Mg, and S) and micronutrients (Zn, Fe, Mn, and Cu), according to Malavolta (1997). Insoluble neutral detergent fiber (INDF) and insoluble acid detergent fiber (IADF) contents were determined following Van Soest et al. (1991) adjusted to a Tecnal TE-149 fiber tester. After INDF determination, the residual fiber was incubated for ADF determination. In vitro dry matter digestibility (DMD) was determined using protocol described in Tilley and Terry (1963), modified by Van Soest et al. (1966), in an Daisy II-ANKOM® incubator (AD II ; Ankom Technology Corporation Fairport, NY, USA), using ruminal fluid from cattle fed with tropical grasses. Other parameters were determined according to Silva and Queiroz (2002).

5.4.4 DNA Extraction and High-Throughput Amplicon Sequencing

DNA extraction from the pasture bulk soil was performed using the standard DNeasy PowerSoil kit protocol (MO BIO Laboratories, Inc.). For plant tissue DNA extraction, cetyltrimethylammonium bromide (CTAB)–based technique (DOYLE; DOYLE, 1987) was used, with specific adjustment on RNAse A concentration depending on the forage type. To access the bovine oral microbiota, DNA extraction from dental biofilm samples was performed with the GenElute Mammalian Genomic DNA Miniprep Kit following manufacturer protocol (Sigma, St. Louis, USA).

Amplification of the V4 region of the 16S rRNA gene for DNA samples was performed using barcoding DNA (CAPORASO et al., 2012) with specific modifications to primer degeneracy (515F - GTGCCAGCMGCCGCGGTAA; 806R - GGACTACHVGGGTWTCTAAT). PCR products were purified and subjected to library preparation and sequencing (251bp x 12bp x 251bp) with Illumina MiSeq technology. Peptide nucleic acid (PNA) PCR clamps were used to suppress plant host plastid and mitochondrial 16S contamination in plant tissue DNA samples (LUNDBERG et al., 2013). Sequencing followed the Earth Microbiome Project protocol for 16S Illumina Amplicon at the Environmental Sample Preparation and Sequencing Facility at Argonne National Laboratory, USA.

5.4.5 qPCR sequencing of 16S rRNA and streptomycin biosynthesis gene (strB1)

A quantitative real-time PCR (qPCR) approach was used to target the 16S rRNA and the *strB1* genes in DNA samples of soil, forage, and cattle. The *strB1* is one of the highly conserved genes in streptomycetes that encode the streptomycin biosynthetic pathway. To target the 16S rRNA gene, the 1055yF (5'-ATGGYTGTCGTCAGCT-3') and 1392R (5'-ACGGGCGGTGTGTAC-3') (RITALAHTI et al., 2006) primers were used. The streptomycin biosynthesis gene was quantified using the primers *strB1F* (5'-ACTACGAGAGCCAGGAGCAGAT-3') and *strB1R* (5'-TGACTCCGAGCTTGGTCAACT-3') (TANAKA; TOKUYAMA; OCHI, 2009). To perform this analysis, we used the sequenced DNA from soils in both forest and pasture systems to evaluate the variations of the abundance of the measured genes addressed the forest-to-pasture conversion. Each reaction mixture (20mL)

containing 1X SsoAdvanced Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc.), primer sets (300 nM each), UltraPure DNase/RNase-free distilled water (Invitrogen), and 2mL of template DNA, were performed using triplicates, and thermocycling reactions were completed using a CFX96 Real-Time PCR (Bio-Rad Laboratories, Inc.). PCR-grade water was included as no-template control samples to account for extraneous nucleic acid contamination. Standard curves were generated using linear regression analysis of synthetic template DNA standards (Integrated DNA Technologies, Iowa, USA) versus their quantification cycles. The synthesized DNA concentration was adjusted to 0.1 ng/mL and 10-fold serial dilutions were prepared ranging from 3.65×10^1 to 3.65×10^8 gene copies per reaction. The thermal cycling protocol was as follows: 2 min at 50°C, 10 min at 95ºC followed by 40 cycles of 15 s at 95ºC for denaturing, 60 s at 58ºC (*strB1*: 30 s at 60 °C for annealing and 30 s at 72 °C for extension). The fluorescence signal was measured at the end of each annealing step.

5.4.6 Sequencing Data Processing

Description found in section 4.4.4.

5.4.7 Statistical Analysis

a) Selection of soil and forage variables

To select the set of abiotic variables that best distinguishes HSL from LSL systems, soil and forage chemical and physical characterizations were submitted to an integrated feature selection method that associates Least Absolute Shrinkage and Selection Operator (LASSO) to Random Forest. A first step performs the selection of variables separately for each method, aiming to generate two subsets with importance scores based on mathematical criteria of each model type. Then, a second step associates the two subsets of scores (RF:LASSO) to build a normalized feature importance list. Finally, only those features with above-average normalized importance (positive scores) were retained for further analysis. All measured abiotic variables were submitted to min-max normalization prior statistical analyses (PATRO; SAHU, 2015). To ensure stability in the final set of selected features, each model was performed with 1000 bootstraps. The functionalities found in the R packages 'glmnet' v.4.1-1 and 'ranger' v.0.12.1 (FRIEDMAN; HASTIE; TIBSHIRANI, 2010; WRIGHT; ZIEGLER, 2017) were accessed to support the method.

b) Metacommunity data analysis

The quality step (filtering, denoising, and the removal of chimeras) on the abundance matrices was used to eliminate low prevalence sequences, as well as *Chloroplast*, *Eukaryota*, and *Mitochondria*. A total of 1,371,914 read counts, divided into 11,470 amplicon sequence variants (ASVs) with 13,450 average counts per sample. Abundances were standardized by the median sequence depth (13,450.5 paired-reads).

To establish the microbial community with the greatest association with the components of the soil-plant-animal continuum in HSL and LSL systems, the set of ASVs belonging to a minimal prevalence of 50% among samples of each component (*core()* function, microbiome R package v.1.12.0) was defined as core microbiota. Seeking greater fidelity for the outputs of abundance rank-based analysis, we used the *format_to_besthit()* function ('microbiomeutilities' R package

v.1.00.16) that fits the best available taxonomy for ASVs, allowing variations in the taxonomical hierarchy in the same list.

Nonmetric multidimensional scaling (NMDS) was performed with the Bray-Curtis distance to visualize (dis)similarities in the community structure of the core microbiota within and between disease severity levels. Subsequently, permutational analysis of variance (PERMANOVA; ANDERSON, 2014) was used to calculate differences in the core microbiota structure of HSL and LSL groups using Hellinger transformed data (LEGENDRE; GALLAGHER, 2001), both with 10,000 permutations. Analyzes were handled exploring the R packages 'phyloseq' v.1.34.0 (MCMURDIE; HOLMES, 2013) and 'vegan' v.2.5-7 (OKSANEN et al., 2016). The indicator value index (IndVal; 'indicspecies' R package v.1.7.9) which combines the mean abundances of a taxon and its frequency of occurrence in all samples was used to ranking those taxon with higher specificity and fidelity (CÁCERES; LEGENDRE, 2009; DUFRÊNE; LEGENDRE, 1997). Correlation heatmap was used to visualize and calculate associations between selected abiotic variables and soil and forage prokaryotes extracted after the differential abundance testing through Random Forest method. The same statistical test was used to rank the most important taxa in the core microbiota of the soil, forage, and cattle components to differentiate the HSL and LSL systems. Analysis and visualization were carried out exploring the functionalities of the 'microeco' R package v.0.4.0 (LIU et al., 2021).

c) Alpha and gamma diversities

Diversity partitioning means that, in a given system, the gamma diversity of all individuals/ASVs found can be divided internally, and within the plot unit (alpha diversity) (DALY; BAETENS; DE BAETS, 2018). Here, we used Hill numbers, which generate effective numbers of equally frequent species for each value of 'q' in a unified framework, which allows the straightforward interpretation and comparison (CHAO; CHIU; JOST, 2014). The order of diversity 'q' attaches different sensitivity to rare species, being: $q = 0$ the most sensitive (species richness); $q = 1$ all individuals are equally weighted (exponential of Shannon's entropy); and $q = 2$ is sensitive to the dominant species (inverse of Simpson index) (JOST, 2006). Because Hill numbers are continuous and have a common unit, they can be portrayed on a single graph as a function of 'q', leading to a "diversity profile" of effective species. Further details can be found in Chao et al. (2014). Analysis was performed using the 'entropart' v.1.6-1 R package (MARCON; HÉRAULT, 2015).

d) Co-occurrence network analysis

A correlation-based network was carried out to verify the topological features of the networks between the microbial communities of the soil-plant-animal continuum, through the abundance matrices of each component. Only significant associations ($p < 0.01$) between ASVs were retained, with the correlation threshold optimized through Random Matrix Theory (RMT)-based method (DENG et al., 2012). The same procedure was performed to build individual networks of each component within each severity level system (HSL and LSL). Finally, high resolution networks (genus level) were applied to verify the topological features regarding the core microbiota among each component within HSL and LSL systems. Correlation analysis were performed using the functions and dependencies found in the 'microeco' R package v.0.4.0 (LIU et al., 2021). Networks (based on the Fruchterman-Reingold layout) and topologies were obtained

through the resources found in the Gephi platform v.0.9.2 (BASTIAN; HEYMANN; JACOMY, 2009).

d) Prediction of metabolic profiles

In addition to providing a taxonomic overview, we used the targeted 16S rRNA sequences to predict community functional profiles, aiming to compare metabolic pathways potentially found among the components of the soil-plant-animal continuum on HSL and LSL systems. Tax4Fun (ASSHAUER et al., 2015), an open-source R package to predict functional profiles on 16S rRNA amplicon sequencing data based on KEGG Orthologs (KO) and SILVA SSU Ref 123 NR, were accessed. We focused on specific metabolic pathway classes within KO (level 2) as such: "Carbohydrate Metabolism", "Biosynthesis of Other Secondary Metabolites", and "Glycan Biosynthesis and Metabolism", for containing a greater number of physiological mechanisms potentially capable of pointing out distinctions between the microbiota associated with soil, forage, and cattle, from systems under low and high severity level of cattle periodontitis. Linear discriminant analysis (LDA) effect size (LEfSe) (SEGATA et al., 2011) was used to test differences in the abundance of predicted functions between HSL and LSL system for each component of the soil-plant-animal continuum.

e) Quantitative real-time PCR (qPCR analysis)

Additionally, the threshold cycle (Ct) of the quantitative real-time PCR was obtained and averaged from triplicate samples**.** Normalized abundances, or the proportions of Bacteria with streptomycin biosynthesis gene (*strB1*), were calculated for soil and forage samples by normalizing *strB1* absolute abundances to the absolute abundances of the Bacteria conserved 16S rRNA gene. To test differences in the number of normalized gene copies between disease severity levels, and the influence of the increases in sum of bases (SB) from forest to pasture soils on *strB1*/16S rRNA proportion, a linear mixed-effect models ('lme4' R package v. 1.1-26), and type II analysis-of-variance/Wald F-test ('car' R package v. 3.0-10) were used after residuals checking.

5.5 RESULTS

5.5.1 Soil and forage abiotic variables differ between high-severity level (HSL) and low-severity level (LSL) pastures, shaping prokaryotes by affinity

After normalization of the importance scores generated by the integrated feature selection method (Random Forest : LASSO), a set of soil chemical and physical variables, as well as bromatological features of the forage, were retained in the final score ranking (Figure 19, Figure). Among the selected edaphic variables, silt:clay ratio (Kruskal-Wallis test, $p < 0.001$; ± 4.4 times), silt content ($p < 0.001$; ± 3.2 times), Cu ($p < 0.001$; ± 2.1 times), CEC eff. ($p < 0.001$; ± 1.6 times), Al ($p = 0.376$; ± 2.5 times), and Na ($p < 0.001$; ± 3.4 times), were higher in LSL pastures. The bromatological variables of greatest importance among LSL pastures were Zn ($p = 0.002$; ± 1.6 times), Mn ($p < 0.001$; ± 2.8 times), Ca ($p = 0.006$; ± 1.1 times), P ($p < 0.001$; ± 1.4 times), insoluble protein (ProtB2) ($p = 0.173$; ± 1.1 times), Mg ($p < 0.001$; ± 1.4 times), and soluble protein (ProtB1) ($p = 1.0$; ± 1.3 times). HSL soils showed higher sand content ($p < 0.001$; ± 7.4 times) and C:N ratio ($p < 0.001$; ± 1.3 times), and the nitrogen-free extract (NFE, $p = 0.040$; ± 1.1 times) was higher in forage leaves.

A high positive correlation (> 60%; $p < 0.001$ after false discovery rate) was observed between *Actinobacteria* (*Thermoleophilia*, *MB-A2-108*), *Acidobacteria* (*Holophagae*), and *Rokubacteria* (*NC10*) and silt content, as well as between *Acidobacteria* (*Holophagae* and *Subgroup_18*) with silt:clay ratio (Figure 20). *Alphaproteobacteria* was highly correlated with sandy texture soils, whereas *Acidobacteria* (*Subgroup 5*) showed a high correlation with Na and effective cation exchange capacity (CEC eff.). *Actinobacteria* (*Thermoleophilia*, *MB-A2-108*), *Acidobacteria* (*Holophagae*), and *Rokubacteria* (*NC10*), were negatively (> - 60%; $p < 0.001$ after false discovery rate) correlated with C:N ratio. Regarding the forage microbiota, *Firmicutes* (*Bacilli*) was positively correlated (> 50%; $p = 0.006$) with protein B2 (ProtB2) and negatively correlated with nitrogen-free extract (NFE, - 61%; $p < 0.001$). *Actinobacteria* was positively correlated with NFE (50%, $p = 0.016$).

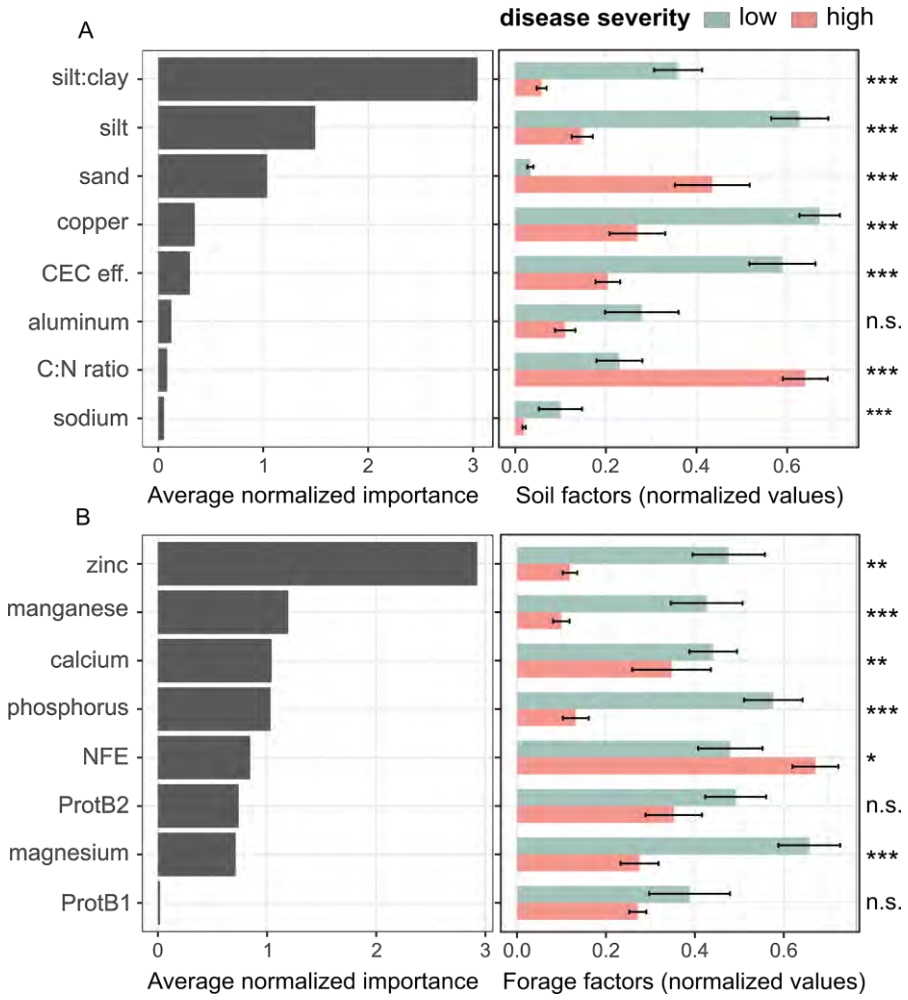

Figure 19. Abiotic variables of A) soil and B) forage selected by the Random Forest: LASSO integrated model. Values subject to min-max normalization prior to analysis of variance; bars indicate the magnitude of each variable in HSL and LSL systems.); (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. not significant. Error bars indicate the ± standard error (SE) (n = 5); CEC eff.: effective cation exchange capacity; NFE: nitrogen-free extract, ProtB2: insoluble protein (intermediate); ProtB1: soluble protein.

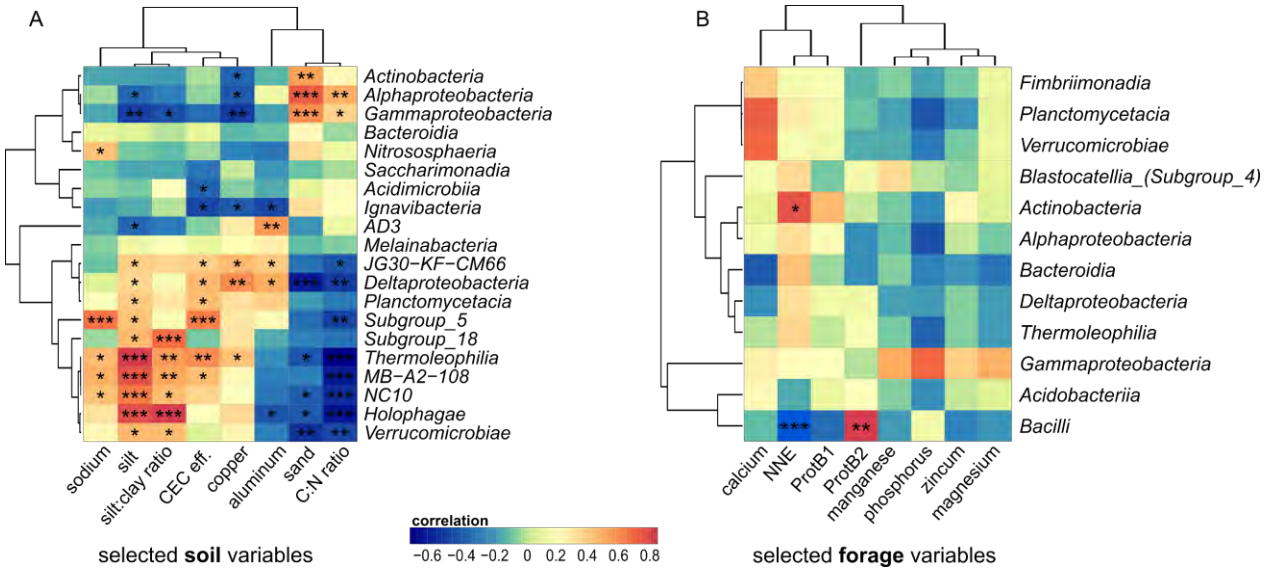

Figure 20. Heatmap analysis for the correlations between selected abiotic variables and the bacterial community. A) soil, B) forage. CEC eff: effective cation exchange capacity; NFE: nitrogen-free extract, ProtB2: insoluble protein (intermediate); ProtB1: soluble protein.

5.5.2 Assessing the structure and diversity of the core community of soil, forage, and cattle in the HSL and LSL systems

To identify the core microbiota, we selected the most prevalent (minimum occurrence threshold in 50% of the samples) ASVs within each component of the metacommunity (i.e., soil, forage, and cattle sequences). After merging the subset of the original bacterial community by category (severity level) within each component, aspects of the community structure were investigated using non-metric multidimensional scaling (NMDS) based on the Bray-Curtis distance (Figure 21). The core microbiota of all the evaluated components reflected different prokaryotic community structure (PERMANOVA; soil: $p < 0.001$, $F = 13.56$; forage: $p = 0.002$, $F = 4.12$; cattle: $p = 0.003$, $F = 2.49$), with evident clustering among samples belonging to the same severity level.

The partitioning of diversity added understanding to the observed differences in core community structure. The alpha diversity, which assigns an average value of the total effective number of species in a given ecosystem (gamma diversity), had a great importance to all the components belonging to the HSL system, with mostly higher values among all the orders of diversity "q" (0 = ASV richness, 1 = "typical" species, 2 = dominant species), except for q = 2 in the soil bacterial core community, which was slightly higher on LSL (Figure 22). However, the gamma diversity attributed to the core microbiota of all HSL components was effectively increased through all orders of diversity. In general, all the components of diversity (0, 1, and 2) were higher in soil, forage, and animals from HSL systems, in both alpha and gamma diversities.

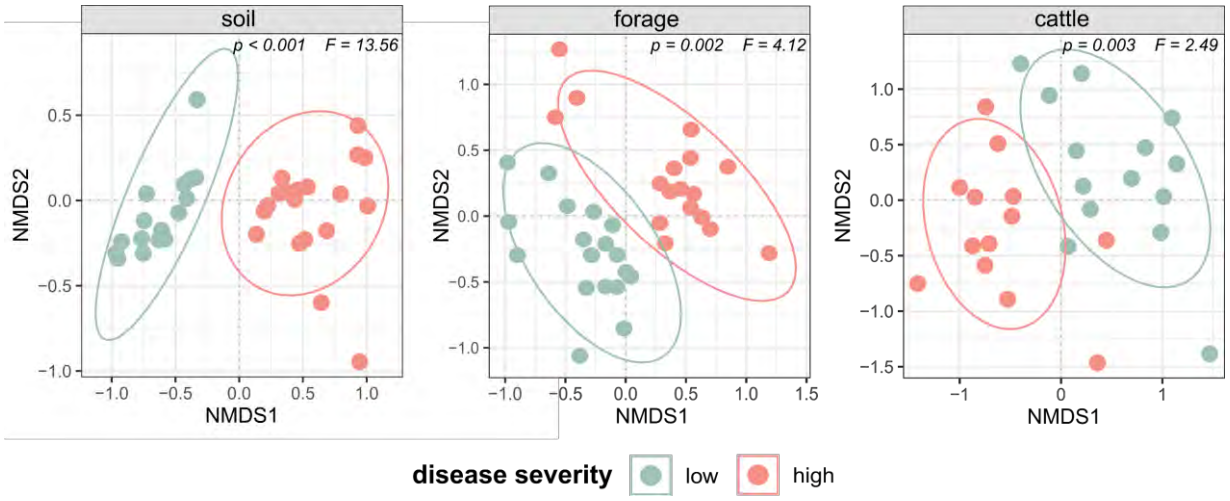

Figure 21. Non-metric multidimensional scale (NMDS) based on Bray-Curtis dissimilarity matrix between samples in normalized ASV abundance data of core microbiota recovered from soil, forage, and cattle. Differences in the structure of the core microbiota were tested statistically by PERMANOVA with 999 permutations.

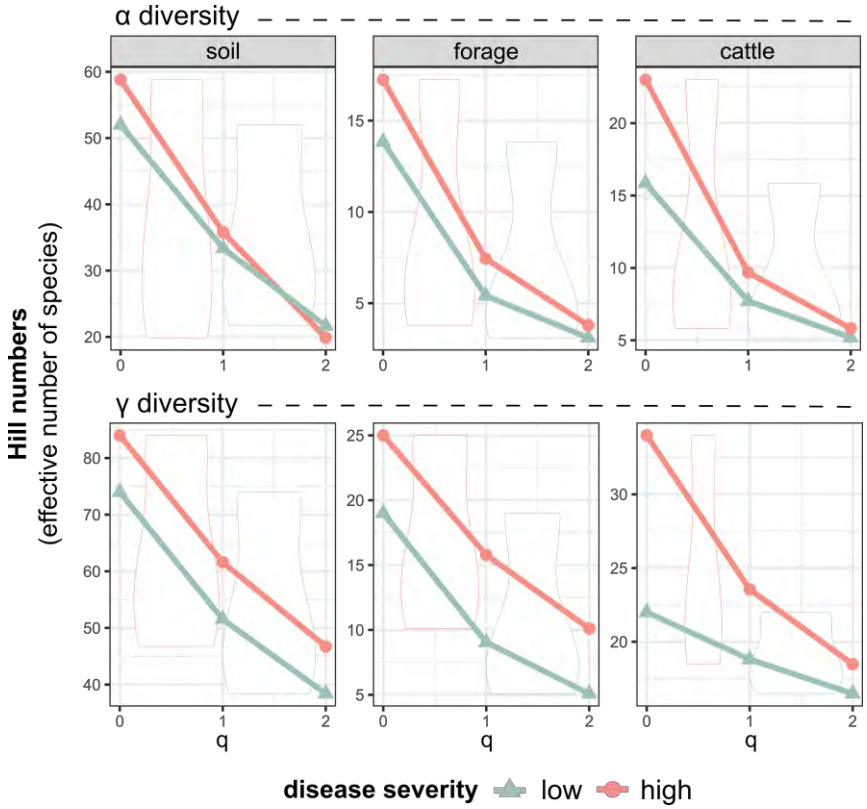

Figure 22. Diversity partitioning analysis. Alpha (local) and gamma (total) diversity and diversity components, with Hill numbers being (q = 0, ASV richness), (q = 1, exponential Shannon entropy for equally weighted ASVs), and (q = 2, inverse of Simpson index for dominant taxa).

5.5.3 Bacterial community signature of HSL and LSL systems

a) Overall core microbial community

The core microbiota of HSL pasture soil is mostly composed by *Proteobacteria* (32.5%), *Acidobacteria* (27.1%), *Actinobacteria* (20%), and *Verrucomicrobia* (13.3%). *Alphaproteobacteria* (*Xanthobacterales*, *Unknown Family*, *Burkholderiaceae*), *Acidobacteria* (*Acidobacterales*, *Acidobacteraceae (Subgroup 1), Acidothermaceae*), *Actinobacteria* (*Streptomycetaceae*), and *Verrucomicrobia* (*Pedosphaeraceae*, *Xiphinematobacteraceae*) were the 10 most prevalent and abundant bacterial families (or higher). The core community of LSL pasture soils include *Proteobacteria* (34.4%), *Acidobacteria* (28%), *Verrucomicrobia* (21.3%), and *Actinobacteria* (15.7%), where the top 10 most prevalent and abundant (IndVal) families (or higher) belong to *Alphaproteobacteria* (*Xanthobacterales*, *Sphingomonadaceae*), *Acidobacteria* (*Acidobacterales*, *Solibacteraceae (Subgroup 3)*, *Acidothermaceae*), *Actinobacteria* (*Solirubrobacteraceae*, *Streptomycetaceae*), *Firmicutes* (*Bacillaceae*), and *Verrucomicrobia* (*Chthoniobacteraceae*, Pedosphaeraceae) (Figure 23). Regarding the prokaryotes in forage microbiota, *Gammaproteobacteria* (*Enterobacteriaceae*), *Alphaproteobacteria* (*Methylobacterium*, *Sphingomonas*, *Aureimonas*) and *Actinobacteria* (*Curtobacterium*) were the main taxa in the core communities of both HSL and LSL forages, albeit with particular importance for *Pseudomonas* (*Gammaproteobacteria*) in forages of LSL, and *Quadrisphaera* (*Actinobacteria*) in forages of HSL. The relative abundance of the core community present in the HSL forage comprises 79.3% of the sequences belonging to the phylum *Proteobacteria*, 18% to *Actinobacteria*, and 2.7% to *Bacteroidetes*. Forages from LSL pastures have 83.6% of the core community by *Proteobacteria*, 12.2% by *Actinobacteria* and 17.6% by Firmicutes.

Finally, regarding the bacterial communities from cattle with HSL, the core microbiota is predominantly composed by *Proteobacteria* (53.6%), *Fusobacteria* (29.2%), *Bacteroidetes* (12.5%), *Firmicutes* (2.8%), and *Actinobacteria* (1.7%), whereas LSL pasture cattle include *Proteobacteria* (82.3%), *Fusobacteria* (6.2%), *Bacteroidetes* (5.4%), *Actinobacteria* (3.9%), and *Firmicutes* (2%). The HSL core cattle community showed a higher number of genera (= 22) with sequences in at least 50% of subgingival biofilm samples than in cattle with LSL (= 18). The genus *Moraxella* (*Gammaproteobacteria*) was the most prevalent and abundant in both systems. however, the core community of HSL cattle had a greater contribution of the genus *Caviibacter* and *Fusobacterium* (*Fusobacteria*), *Porphyromonas* and *Bergeyella* (*Bacteroidetes*), whilst cattle under LSL had a greater predominance of *Pseudomonas*, *Alysiella*, *Haemophilus*, and *Escherichia*/*Shingella* (*Gammaproteobacteria*).

b) Differential abundance and indicator species (IndVal) of the representative core microbiota taxa

To disentangle the linkages between the core microbiota and levels of disease severity, a Random Forest approach was used to identify the main taxa contributing to each evaluated component (soil, forage, and cattle) (Figure 23). Based on the relative abundance of each core microbiota, we identify 65, 17, and 27 biomarkers (genus level) among soil, forage, and cattle samples, respectively, capable to represent the enrichment/depletion in HSL systems.

Among the top important core taxa in soil, we found a depletion of *Gaiellales* (*Actinobacteria*), *Bacillus* (*Firmicutes*), *Haliangium* (*Deltaproteobacteria*), *Candidatus Solibacter*

(*Acidobacteria*), *Candidatus Udaeobacter* (*Verrucomicrobia*), and *Rhodomicrobium* (*Alphaproteobacteria*), and enrichment of *HSB_OF53-F07* (*Ktedonobacteria*), *Burkholderia-Caballeronia-Paraburkholderia* and *Leifsonia* (*Gammaproteobacteria*), *Occallatibacter* and *Bryobacter* (*Acidobacteria*), and *Elsterales* (*Alphaproteobacteria*).

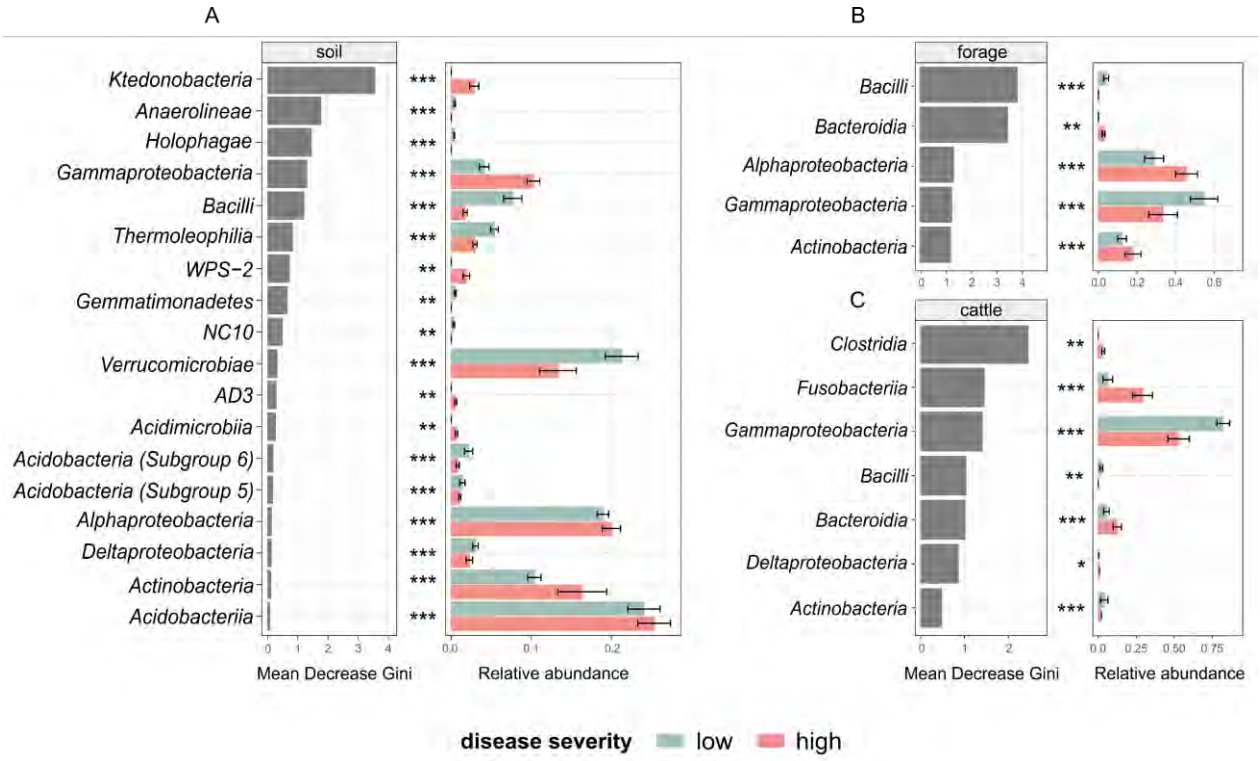

Figure 23. Discriminant abundance analysis based on bacterial classes by disease severity level. A) soil, B) forage, C) cattle; Bacterial classes were ranked decreasingly based on Random Forest's Gini importance value; (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. not significant.

In the top forage core microbiota from HSL systems it was found a depletion of *Bacillus* (*Firmicutes*), *Pseudomonas*, *Enterobacteriaceae* (*Gammaproteobacteria*), and *Kineococcus* (*Actinobacteria*), and enrichment by *Spirosoma* (*Bacteroidetes*), *Xanthomonas* (*Gammaproteobacteria*), *Geodermaphilus* (*Actinobacteria*), *Acetobacteraceae*, and *Sphingomonas* (*Alphaproteobacteria*). The top important cattle core microbiota was depleted by a group of *Gammaproteobacteria* (*Yersinia*, *Alysiella*, *Actinobacillus*, *Acinetobacter*, *Escherichia*/*Shigella*, *Pseudomonas*), *Streptococcus* (*Firmicutes*), *Corynebacterium* (*Actinobacteria*), whereas *Bacteroidales* (*Bacteroidetes*), *Fusobacterium*, *Caviibacter* (*Fusobacteria*), *Neisseriaceae* (*Gammaproteobacteria*), *Desulfovibrio* (*Deltaproteobacteria*), and *Peptoanaerobacter* (*Firmicutes*) were found with significative enrichment in diseased cattle. Overall, the class *Bacilli* was relatively more abundant among soil, forage, and cattle from LSL systems. Forage and cattle from LSL system shared importance for the higher relative abundance of the class *Gammaproteobacteria*, being *Bacteroidia* more pronounced on forage and cattle under HSL. A set of 10 indicator species (IndVal) were selected by their specificity (large mean relative abundance within group) and fidelity (presence in most samples of the same group) taxa, are

displayed on Table 2. Considering the most important features selected by both Random Forest and IndVal, only the genus *Leifsonia* (*Actinobacteria*) and *ADurb.Bin063-1* (*Verrucomicrobia*) were considered as highly representative in the soil core microbiota from HSL and LSL system, respectively.

Table 2. Rank of the 10 most important bacterial genera in soil, forage, and cattle as indicators of HSL and LSL systems of cattle periodontitis. Ranking is based on the IndValue and high statistical significance of each taxa.

Soil			Forage			Cattle		
High Severity (HSL)								
	IndVal	**p-value**		**IndVal**	**p-value**		**IndVal**	**p-value**
Leifsonia	0.727	<0.001	*Siporosoma*	0.680	<0,001	*Caviibacter*	0.674	<0.001
Occallatibacter	0.683	<0.001	*Hymenobacter*	0.602	<0,001	*Actinomyces*	0.668	<0.001
Dyella	0.652	<0.001	*Herbiconiux*	0.577	<0,001	*Campylobacter*	0.637	<0.001
Domibacillus	0.637	<0.001	*Herbaspirillum*	0.559	<0,001	*Porphyromonas*	0.632	<0.001
X1921.3	0.634	<0.001	*Xanthomonas*	0.552	<0,001	*Proteocatella*	0.618	<0.001
Kutzneria	0.632	<0.001	*Roeomonas*	0.544	<0,001	*Bergeyella*	0.613	<0.001
Sinomonas	0.626	<0.001	*Melittangium*	0.508	<0,001	*Bibersteinia*	0.613	<0.001
Rhodoplanes	0.620	<0.001	*Blastocatella*	0.450	<0,001	*Capnocytophaga*	0.613	<0.001
Roseiarcus	0.620	<0.001	*Psychroglaciecola*	0.424	<0,0015	*Filifactor*	0.558	<0.001
Acidothermus	0.619	<0.001	*Actinomycetospora*	0.423	<0,001	*Peptoanaerobacter*	0.550	<0.001
Low Severity (LSL)								
	IndVal	**p-value**		**IndVal**	**p-value**		**IndVal**	**p-value**
Luedemannella	0.805	<0.001	*Curtobacterium*	0.649	<0.001	*Ruminococcaceae NK4A214*	0.684	<0.001
Anaeromyxobacter	0.711	<0.001	*Aureimonas*	0.624	<0.001	*Corynebacterium*	0.667	<0.001
X1921.2	0.688	<0.001	*Novosphingobium*	0.578	<0.001	*Alysiella*	0.663	<0.001
Aquisfaera	0.685	<0.001	*Kineococcus*	0.501	<0.001	*Yersinia*	0.661	<0.001
Gemmata	0.671	<0.001	*Methylocella*	0.493	<0.001	*Streptococcus*	0.651	<0.001
ADurb.Bin063.1	0.657	<0.001	*Neorhizobium*	0.480	<0.001	*Moraxella*	0.632	<0.001
Rhodomicrobium	0.657	<0.001	*X1174.901.12*	0.431	<0.001	*Ruminococcus*	0.620	<0.001
Reyranella	0.648	<0.001	*Larkinella*	0.360	0,0064	*Haemophilus*	0.620	<0.001
Candidatus Xiphinematobacter	0.645	<0.001	*Enterococcus*	0.347	0.0101	*Christensenellaceae R.7*	0.614	<0.001
Ellin6067	0.645	<0.001	*Methylosinus*	0.320	0.0161	*Ruminococcaceae UCG.010*	0.614	<0.001

For the forage core microbiota, *Spirosoma* (*Bacteroidetes*), *Xanthomonas* (*Gammaproteobacteria*), *Herbiconiux*, and *Actinomycetospora* (*Actinobacteria*), were found as highly representative/enriched features of HSL pasture, while *Curtobacterium*, *Kineococcus* (*Actinobacteria*), *Aureimonas*, *Novosphingobium*, and *Neorhizobium* (*Alphaproteobacteria*) were consistently depleted. Finally, the genus *Caviibacter* (*Fusobacteria*), *Actinomyces* (*Actinobacteria*), *Porphyromonas*, *Bergeyella*, *Capnocytophaga* (*Bacteroidetes*), *Proteocatella*, *Peptoanaerobacter* (*Firmicutes*), and *Bibersteinia* (*Gammaproteobacteria*), were the most relevant in terms of specificity and fidelity, indicating their enrichment in the cattle core microbiota from HSL system. *Corynebacterium* (*Actinobacteria*), *Alysiella*, *Yersinia*, *Moraxella*, *Haemophilus* (*Gammaproteobacteria*), and *Streptococcus* (*Firmicutes*), were considered representative in cattle core microbiota from LSL system.

5.5.4 Co-occurrence network of the prokaryotic metacommunity among HSL and LSL systems

We next calculated a set of network-level topological features to identify the degree of co-occurrence among the global (non-core) metacommunity, which encompasses the whole ASVs (after filtering quality) of each evaluated component (i.e., soil, forage, and cattle) (Figure 24). Overall, the authority, bridging coefficient, modularity, and page rank features were all higher in the HSL metacommunity, whereas the degree (weighted), betweenness, clustering, and closeness centrality were higher in the LSL metacommunity. Similar features were observed in each component separately (Figure 24), especially regarding the higher modularity classes among HSL components.

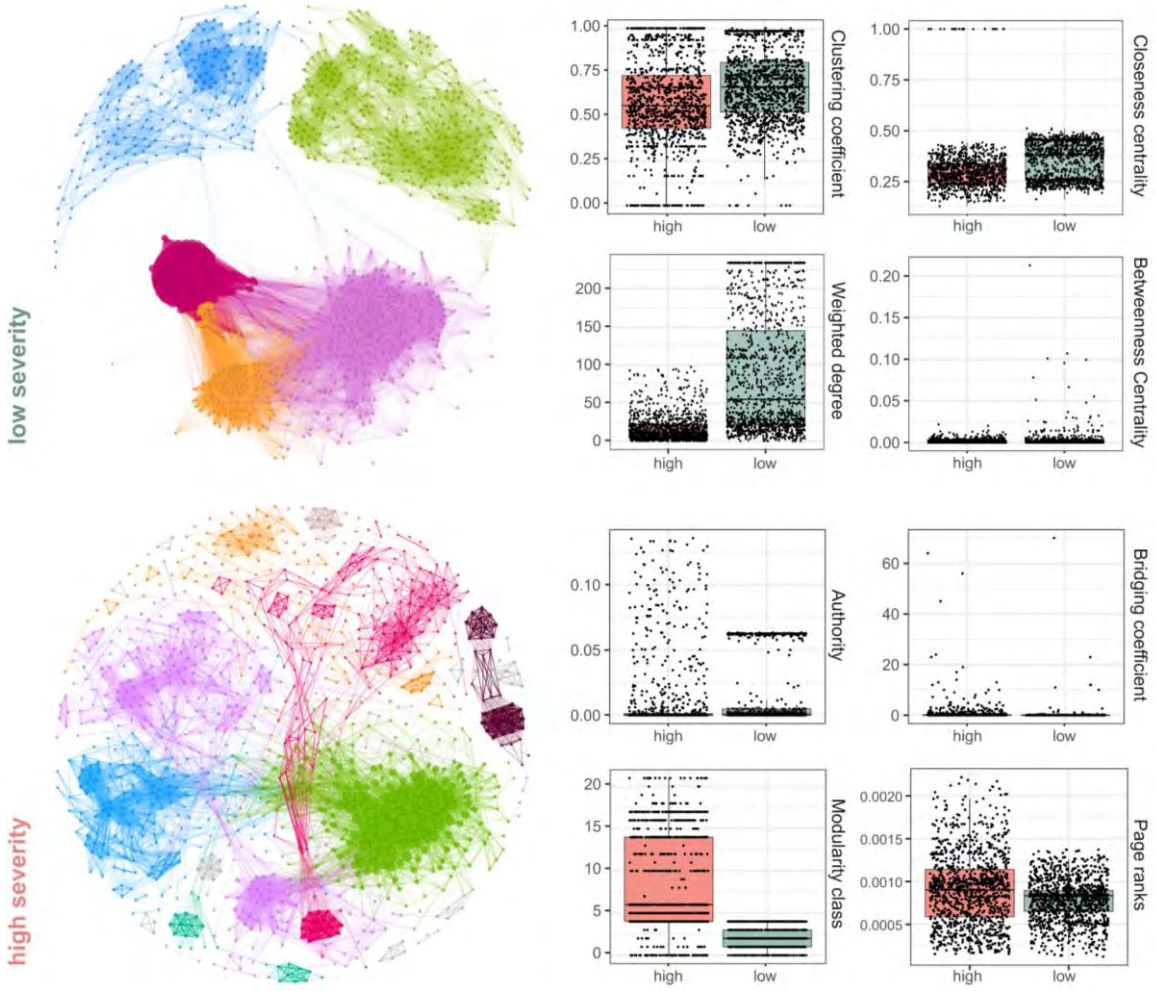

Figure 24. Global co-occurrence network of the soil-plant-animal continuum. Networks established from Spearman correlation between abundance of ASVs from soil, forage, and cattle components. Only significant associations ($p < 0.01$) between ASVs were retained; Correlation threshold optimized (LSL = 0.53; HSL = 0.69) using the Random Matrix Theory (RMT)-based method. Colors represent a module, which can be considered a subcommunity.

a) Keystone taxa of the core microbiota as a high-resolution approach to define healthy and disease-susceptible components

Subsequently, the analysis was applied individually to each core microbiota community to identify keystone taxa that are possibly regulating the main ecological processes within each component, especially those representatives of pastures classified as HSL of the disease (Figure 25).

The co-occurrence network for the soil core community under LSL system had 50.08% of positive correlations. *Acidobacteriia* (18.18%) and *Alphaproteobacteria* (18.18%) were the bacterial classes with the highest amount of highly correlated ASVs, followed by *Verrucomicrobia* (15.15%) and *Bacilli* (10.61%). The top values of betweenness centrality (> 0.06) were observed for ASVs belonging to the taxa *Bradyrhizobium*, *Bacillaceae*, *Mycobacterium*, and *Xanthobacteraceae*. The core community of HSL soils showed predominantly (91.8%) of positive

correlations between ASVs, mostly by *Acidobacteriia* (31.03%), *Verrucomicrobia* (17.24%), and *Alphaproteobacteria* (10.34%). ASVs belonging to *Conexibacter* (*Thermoleophilia*), *HSB_OF53-F07* (*Ktedonobacteria*), and *WPS-2* had a higher betweenness centrality in the network (> 0.30). The co-occurrence analysis for ASVs associated with core microbiota of the forage under HSL showed a strong positive association (92.47%) between the genera *Sphingomonas* (*Alphaproteobacteria*) and *Spirosoma* (*Bacteroidia*), despite the highest weighted degree. Overall, when considered only strong correlations (≥ ± 60%), the network was structured by *Alphaproteobacteria* (61.9%), *Gammaproteobacteria* (14.29%), *Actinobacteria* (14.29%), and *Bacterodia* (9.52%), with 97.22% of positive edges. Forage under LSL had the highest positive taxon-taxon association (79.67%) occurring between ASVs belonging to *Pseudomonas* (*Gammaproteobacteria*) and *Methylobacterium* (*Alphaproteobacteria*). Strong correlated nodes belong to *Alphaproteobacteria* (53.85%), *Actinobacteria* (30.77%), and *Gammaproteobacteria* (15.38%), with 92.31% of positive edges.

The co-occurrence network for the cattle core microbiota under HSL has most part of the nodes referring to *Gammaproteobacteria* (42.42%), *Bacterodia* (24.24%), and *Fusobacteria* (15.15), with the remaining ASVs distributed among the classes *Clostridia*, *Actinobacteria* and *Deltaproteobacteria*. The strongest association (93%) was attributed to ASVs belonging to the genus *Porphyromonas* and *Fusobacterium*, which also had the highest eigenvector centrality (1.0 and 0.84, respectively). Furthermore, ASVs assigned to the genus *Peptoanaerobacter* and *Lautropia* are among those with the highest weighted degree. Finally, the network of the cattle core microbiota under LSL has 72.22% of the highly correlated ASVs belonging to *Gammaproteobacteria*, and 11.11% to *Actinobacteria*. *Bacteriodia* and *Fusobacteriia* account for 16.67% of the total nodes. The strongest correlation (93.9%) was attributed between *Bibersteinia* (*Gammaproteobacteria*) and *Bergeyella* (*Flavobacteriia*), and also was observed that ASVs linked to the genera *Neisseria* (*Betaproteobacteria*), *Moraxella* (*Gammaproteobacteria*), and *Corynebacterium* (*Actinobacteria*) exert greater eigenvector centrality in the co-occurrence network.

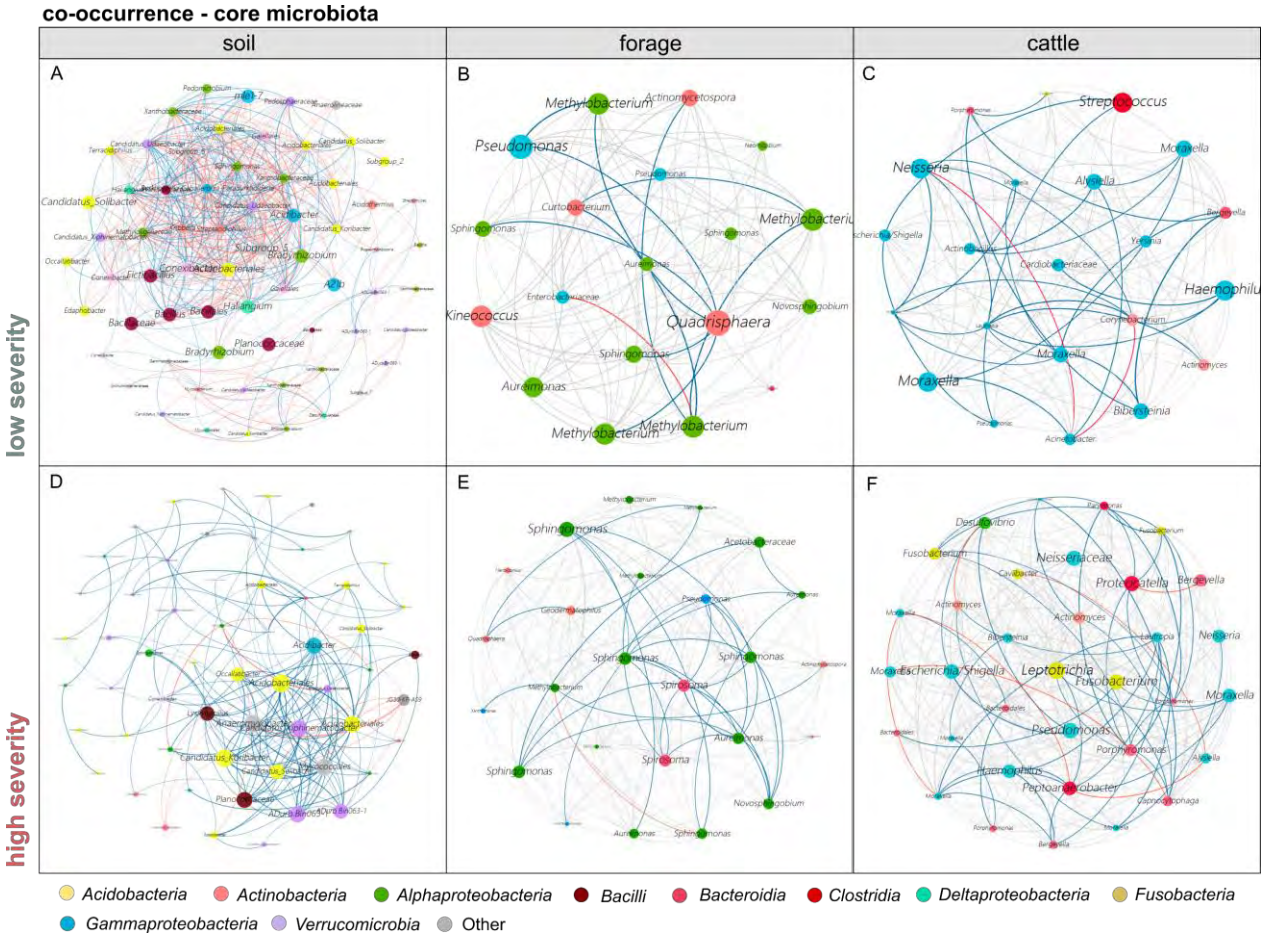

Figure 25. Co-occurrence network for the core community of soil, forage, and cattle from HSL and LSL systems. Blue lines refer to positive correlations and red to negative ones. Co-occurrence networks from the abundance matrix of soil, forage, and cattle prokaryotes from the HSL and LSL systems. Networks were constructed from taxa that showed at least one strong (Spearman; ≥ 0.70) and statistically significant ($p < 0.001$) correlation.

5.5.5 Differences in predicted metabolic pathways of the bacterial metacommunity on HSL and LSL systems

Overall, LDA effect size (LEfSe) outputs highlight that among the biosynthesis of other secondary metabolites pathways, the beta-lactam resistance (KO01501), biosynthesis of indole alkaloid (KO00901), isoquinoline alkaloid (KO00950), novobiocin (KO00401), penicillin and cephalosporin (KO00311) were discriminately abundant among the soil, forage, and cattle components from LSL system (Figure 26).

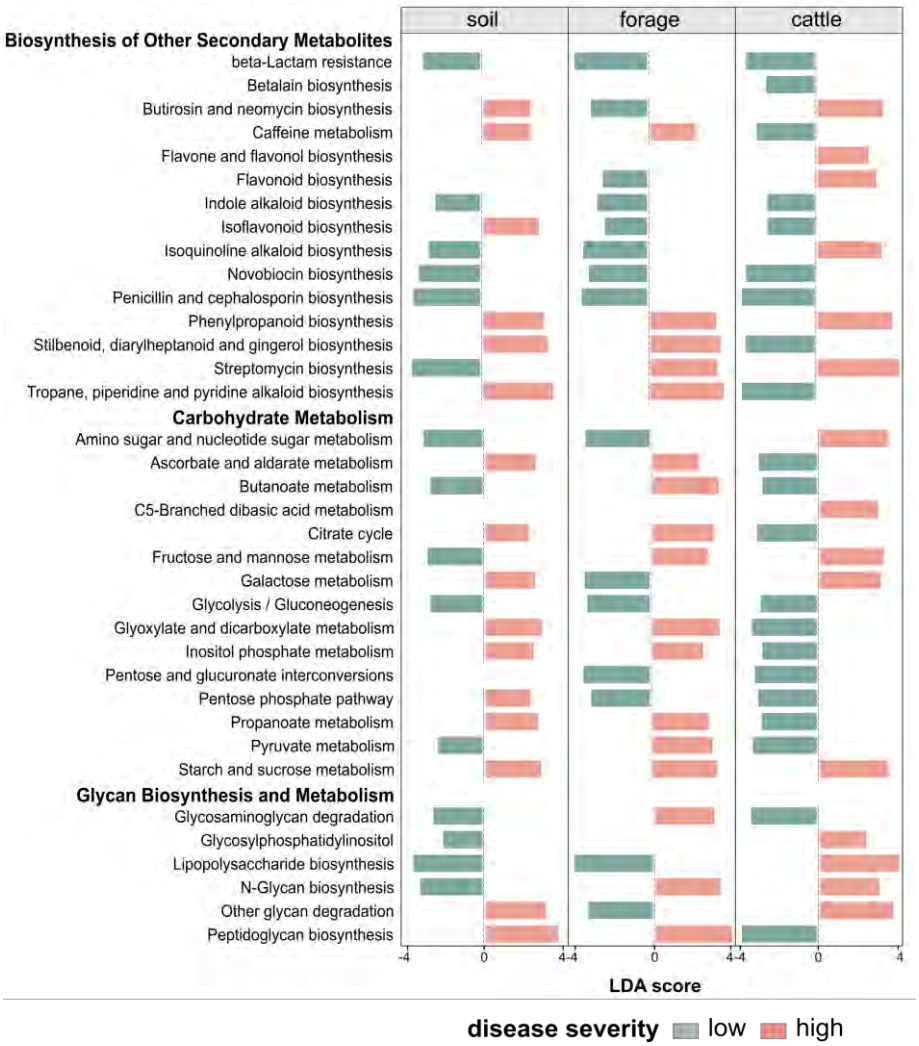

Figure 26. Differences in predicted metabolic pathways for the soil, forage, and cattle bacterial metacommunity of the HSL and LSL systems. Linear discriminant analysis effect size (LDA LEfSe) was used to test the difference in predicted function abundance.

Oppositely, the HSL system components obtained greater enrichment of phenylpropanoid biosynthesis (KO00940) genes, as well as for streptomycin (KO00521) in forage and cattle. The enrichment of the carbohydrate metabolism pathways was predicted among soils and forage of HSL pastures, with starch and sucrose metabolism (KO00500) being found in greater abundance also in cattle samples. Although the low gene abundance for this category, HSL cattle were higher in abundance for amino sugar and nucleotide sugar metabolism (KO00520), also including C5-Branched dibasic acid (KO00660), fructose and mannose (KO00051), and galactose metabolism (KO00052).

Soils from disease-susceptible pastures were depleted of the predicted genes for glycosaminoglycan degradation (KO00531), glycosylphosphatidylinositol (GPI, KO00563), in addition to lipopolysaccharide (KO00540) and N-glycan biosynthesis (KO00510). Other glycan degradation (KO00511) and peptidoglycan biosynthesis (KO00550) were enriched in this system. Cattle on HSL systems evidenced enrichment of 4 of 6 genes within the glycan biosynthesis and

metabolism group, with higher LDA scores to lipopolysaccharide biosynthesis (= 4.0), and other glycan degradation (= 3.77).

5.5.6 Relative abundance of the streptomycin biosynthesis gene (strB1) in the components of the soil-plant-animal continuum

The quantitative PCR for the target gene strB1 and 16S rRNA showed that, in general, the relative abundance of gene copies is higher in all components of the soil-plant-animal continuum on HSL systems (Figure 27).

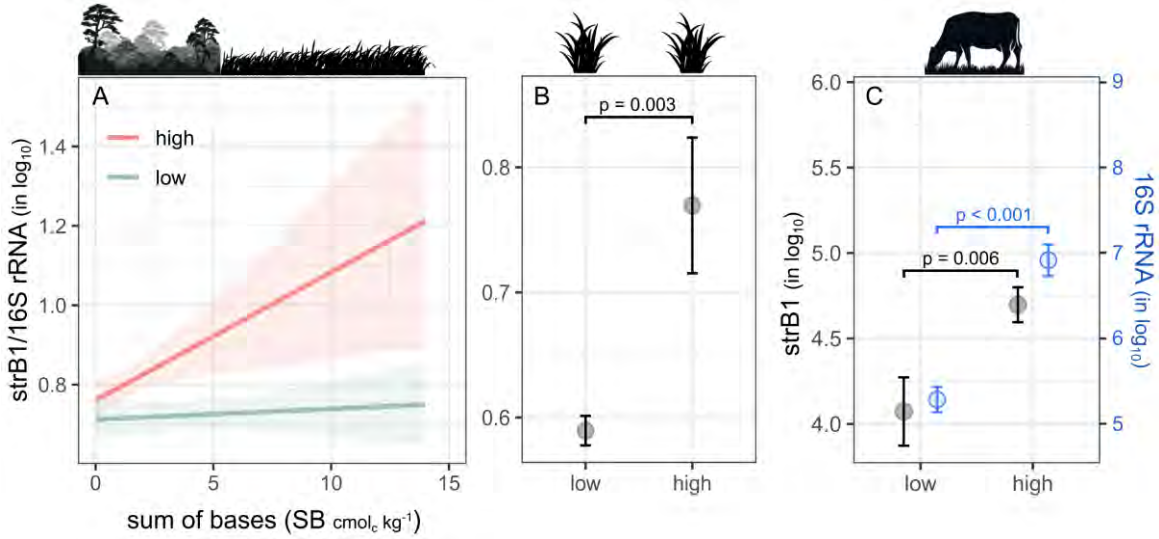

Figure 27. Differences in the relative abundance of the streptomycin biosynthesis gene (strB1) for the components of the soil-plant-animal continuum of the HSL and LSL systems. A) Predicted linear correlation for the increase in soil fertility (represented by the sum of soil bases) and relative abundance of strB1/16S rRNA considering forest and pasture soil samples; B) total difference in relative abundance of strB1/16S rRNA in forage, and C) total difference in relative abundance of strB1 and 16S rRNA in subgingival biofilm samples from cattle.

Encompassing soil samples from both forests and pastures, we verified that the general trend of strB1/16S rRNA gene copies proportion was higher in HSL systems than LSL ($F = 12.62$, $p < 0.001$). Moreover, the statistical interaction of soil sum of bases and disease severity was significant ($F = 4.59$, $p = 0.036$). Streptomycin:16S rRNA gene copies of soils from HSL systems followed a significantly positive correlation with the sum of bases (SB), which increases from forest to pasture soils (Figure 27A). The strB1/16S rRNA proportion was higher in pasture soils ($F = 6.85$, $p = 0.013$) and in forages ($F = 10.14$, $p = 0.003$; Figure 27B) from HSL systems, suggesting a greater capacity of production of this antibiotic by Actinobacteria, especially of the genus Streptomyces spp. Finally, the ingestion of forage and soil by cattle is considered a possible way for microbial enrichment and associated metabolites, besides the metabolic activity of the oral microbiota. Thus, we present the individual quantification values for strB1 and 16S rRNA genes. The abundance of both genes was significantly higher (strB1: $F = 9.07$, $p = 0.006$; 16S rRNA: $F = 37.08$, $p < 0.001$) in subgingival biofilm DNA samples from cattle with periodontal disease (Figure 27C).

5.6 DISCUSSION

Since the 1960s, the pioneering research by Dr. Jürgen Döbereiner and his team has provided observational, quantitative, and qualitative evidence that consistently indicates the pathogenesis of periodontitis in ruminants, previously called as “swollen face disease” (“cara-inchada dos bovinos - CIb” *in Portuguese*), as a multifactorial disease caused by complexes of microorganisms. Internationally, studies by West and Spence (2000) reported as "broken-mouth disease" also evidenced similar clinical picture of the animals, in line with this suggested multifactorial behavior of the periodontal disease. Symptomatically, the disease manifests itself by the destruction of periodontal tissues, loosening, and shedding of cheek teeth, which turns chewing difficult, causing severe weight loss in more chronic cases (DÖBEREINER et al., 2000). Reported as a response to changes in the soil environment, the onset of the periodontal disease is, particularly, a suspected consequence of the forest-to-pasture conversion or pasture renewal as well. Changes in natural or long-term environmentally stable edaphic properties (e.g., increase in pH and base saturation, physical and hydraulic alterations due to the pasture introduction), were considered important factors to explain differences in susceptibility of some farms to the onset of the disease than others. Furthermore, the spatial heterogeneity of soil pedogenetic factors would influence, in part, the distribution of disease susceptibility in soils and forage (DIGNAM et al., 2016; RAAIJMAKERS; MAZZOLA, 2012). Thus, it suggests that pedogenetic process and local environmental factors play important role in this *train of thought,* being important drivers to be considered, rather than mineral deficiency or nutritional imbalance, as previously suggested (ROSA; DÖBEREINER, 1994). However, due to limitations in technology, especially regarding the assessment to the non-cultivated portion of the microbiota, a more robust investigation has been temporarily impeded. Here, we intend to develop and discuss a multidisciplinary approach which integrated both *in silico* molecular biology and environmental data, aiming to contribute to a better understanding of the environmental drivers that possibly culminate in the triggering of periodontal disease in cattle, from an ecosystem perspective.

Initially, we found that among the selected abiotic factors by the integrated model (Random Forest: LASSO), the pastures with low severity level of the disease (LSL) showed higher quality in terms of soil fertility and plant nutrition. Oppositely, the forages with lower macro and micromineral contents were predominant in disease-susceptible pastures (HSL), in addition to higher values of soil C:N ratio, whereby suggesting a lower overall nutritional balance of the system itself. This is possibly due to the high mineralization rate and leaching of the remaining organic matter and nitrogen immobilization by microbial biomass, associated with the pedogenesis of the soils predominantly found in those areas. According to Holmes et al. (2021), the quality of forage may be expected to affect the severity and incidence of periodontal diseases.

Interestingly, the zinc (Zn) content was the top variable selected among the measured forage factors, being found almost two times higher in LSL systems. Also, copper (Cu) was the most important chemical variable selected in the final integrated model for soil abiotic factor. The higher content of soil Cu and Zn in forage of LSL pastures highlights one evidence to be further explored, since those microminerals play an important role in animal health, avoiding problems such as anemia, osteodystrophy, decreased resistance to infectious disease, and diarrhea, often associated with the lack of these elements (TESSMAN et al., 2001). Zinc deficiency is reported as a potential risk factor for oral and periodontal diseases (ORBAK et al., 2007; UWITONZE et al., 2020). Souza et al. (1985) tested the possible effect of mineral supplementation in calves with periodontal disease, and the results pointed out that high Cu supplementation in association with cottonseed

meal increased the animal performance, such as: less mortality, better mineral intake, and higher weight gain in comparison to non-treated animals. Moreover, the reported bromatological analysis indicated adequate amounts of Ca, Mg, K, Fe, Mn, which is in line with our results regarding the higher macro and micromineral content in systems with lower severity level of the disease. In general, among several functions, Zn play important role in plant defense, and its deficiency has been reported to affect the response of a specific host to aphids and pathogens (CABOT et al., 2019). Cu-based antimicrobial compound is used worldwide to crop protection, both for control of bacterial diseases and several fungi and oomycetes, due its high toxicity to plant pathogens, besides the low cost and chemical compounds stability (LAMICHHANE et al., 2018). Furthermore, the role of Cu in enhancing plant abiotic stress tolerance is well described, due to some functions linked to plant metabolism such as the structural strengthening of the cell wall (HASANUZZAMAN et al., 2017).

Ruminants ingest an estimated amount of 2 kg soil day^{-1} depending on the pasture system (ATTWOOD et al., 2019). The relevance of soil ingestion implies different consequences, as such, the abrasiveness of the soil particles in animal dentition, being reported as a significant contributor to teeth wear, which reduces the productive lifetime of ewes (ABRAHAMS; STEIGMAJER, 2003). Soil ingestion could also carry trace elements, minerals (e.g., copper), and other metabolites adhered to clay particles (BERESFORD; HOWARD, 1991). However, cases of periodontitis have already been detected in calves breastfed by cows with periodontitis, even when calves were fed forage from an area considered free of the disease (DÖBEREINER; ROSA; LAZZARI, 1987). Thus, considering the evolution of knowledge about the etiology, epidemiology, and pathogenesis of oral diseases, we did not explore the relationship between abrasiveness by soil particles and the worsening of periodontitis. Nevertheless, although it may not be a critical factor to the onset of the disease, this mechanism should not be ignored in the general interpretation of the infection process. In our results, the sand content was the third most important soil variable in the integrated model, and it was significantly higher in disease-susceptible pasture soils.

According to Peixoto et al. (2021), improving animal health requires the integration of knowledge on microbial distribution and diversity, as well as its pathogenicity, symbiotic mechanisms, and interactions between microbial populations. Dysbiosis, or imbalance of the healthy microbiome, is defined as any deviation in community structure of the resident microbiome of healthy individuals (SCANNAPIECO; DONGARI-BAGTZOGLOU, 2021). A dysbiotic microbiota possibly reflects the conditions of the host's disease and may manifest itself through a decrease in the microbiome stability and/or an increase in its taxonomic variability (KUMAR, 2021). In our study, observing the topologies of the global network which combines ASVs from soil, forage, and cattle microbiota, it was possible to visualize that the co-occurrences associated to LSL systems were better structured across few, but, highly stable and connected modules (i.e., higher clustering coefficient and closeness centrality). Oppositely, in comparison to what has been mentioned above, the global network for HSL systems has its structure based on a greater number of short and highly interconnected modules (i.e., higher page ranks). The main features suggest that the soil-plant-animal continuum of disease-susceptible systems reflects a disrupted prokaryotic metacommunity network, as a result of the lack of keystone taxa, possibly due the effect of the disturbances in the original soil conditions. Moreover, with consequences on forage quality, which possibly enriches the cattle subgingival biofilm with a more stressed/disturbed microbiome.

These observed features were better explored through the assessment of the core microbiota. Our analysis found significative differences between HSL with LSL systems in terms of prokaryotic community structure among all components of the soil-plant-animal continuum. It

suggests that the edaphic environment from disease-susceptible pastures is probably more responsive to land-use intensification, likewise, by the inherent edaphoclimatic and environmental conditions. This process seems to trigger a cycle of chain transformations, which affects the chemical composition of forages and their associated microbial community, such as endophytes and phyllosphere. Consequently, the oral microbiota of ruminants is possibly attacked after ingestion of the plants, which carries a sort of disturbed microbiota and its associated metabolites and antibiotic resistance/biosynthesis genes. A recent review by Scannapieco et al. (2021) points out that, in human periodontitis, the infection process results in increased bacterial diversity compared with periodontal health. In the same way, this trend was also found in bovine oral microbiota (BORSANELLI et al., 2018). Through the partitioning of diversity approach, we went further on this issue, highlighting that the alpha and gamma microbial diversity are significatively higher in all the components of the soil-plant-animal continuum from disease-susceptible systems. The set of disease triggers seem to exert greater stress on the associated prokaryotic community, increasing the richness, diversity, and dominance (here expressed as Hill numbers 0, 1, and 2) of ASVs in those components. The increased diversity in the pastures of the HSL system reinforces the interpretation provided by the co-occurrence analysis, and is in line with the statistical differences observed in microbial community structure between the HSL and LSL systems.

Notwithstanding, we seek to understand how those different forms of structuring and metacommunity dynamics can also define keystone taxa, and the possible role of ecological functions they play in disease-susceptible systems compared to healthy ones. In recent years, especial attention has been given to research aimed at understanding the role of microorganisms in disease suppression. These studies have aimed to discover new functions of microbiomes, as well as plant-soil feedbacks that enhance the recruitment of microorganisms capable of alleviating environmental stress, and reduce pathologies and the demand for agrochemicals through management practices that increase ecosystem resilience (BERG; KRAUSE; MENDES, 2015; RAAIJMAKERS; MAZZOLA, 2016). Some organisms have been recognized as potential taxonomic indicator of disease suppressive function, such as some *Bacillus* (*Firmicutes*) and *Pseudomonas* (*Gammaproteobacteria*) strains, which are well-characterized bacterial groups known to produce a broad range of antibiotics (RAAIJMAKERS; MAZZOLA, 2012; SUN et al., 2021). Regarding the greatest important prokaryotes in LSL systems, the class *Bacilli* was observed among all the evaluated components, and *Gammaproteobacteria* (including *Pseudomonas* spp.) was significantly higher in forage and cattle core microbiota. These bacterial groups have been also reported as indicators of healthy oral microbiota, both in humans (XU; GUNSOLLEY, 2014) and in cattle (BORSANELLI et al., 2018). Moreover, those taxa, which can be both soilborne and/or endophytes (HARDOIM; VAN OVERBEEK; VAN ELSAS, 2008), exhibit antibacterial and antifungal activity against phytopathogens through secretory products (RAAIJMAKERS; MAZZOLA, 2012), however, this behavior is highly dependent on the nutrient availability conditions of the substrate (SUN et al., 2021). Otherwise, some typically anaerobic taxa such as *Clostridiales* and *Bacteroidetes* are predominantly found in DNA samples from disease-susceptible systems. Those biomarkers were also found in the core microbiota from HSL systems, corroborating the microbial signature that differentiates healthy from diseased cattle.

As pointed out by Banerjee et al. (2018), linking microbial community structure to function is one of the central objectives in microbial ecology. Likewise, it is necessary to include patterns of co-occurrence and keystone taxa to tackle a better understanding about the ecosystem processes under investigation. The same author highlights that those keystone taxa, which play role in community functioning, must also be part of the core microbiome and be consistently found in the

environment. Hence, defining such core microbiome is critical to understand the assemblage, connectivity, and stability of microbial communities (SHADE et al., 2012). In our study, most taxa highlighted as biomarkers by the differential abundance analysis and indicator species (IndVal) were equally important considering the measured network interactions. For example, in disease-susceptible systems, ASVs associated with the forage core microbiota showed a strong positive association (> 90%) between the genus *Sphingomonas* (*Alphaproteobacteria*) and *Spirosoma* (*Bacteroidia*). In cattle samples, the strongest association was attributed between ASVs belonging to the genus *Porphyromonas* (*Bacteroidetes*) and *Fusobacterium* (*Fusobacteria*), previously documented biomarkers of diseased oral microbiota (BORSANELLI et al., 2018; SILVA et al., 2019). We also showed that the soil core community of LSL systems has approximately half of the interactions/correlations as antagonistic relationships, which seems to corroborate the lower ASV dominance evidenced in the diversity partitioning analysis. Otherwise, pasture soils from disease-susceptible systems showed more than 90% of positive correlation among the total connections in the network using the core community. Considering the microbial plasticity in response to changes in the soil environment, a better understanding of the mechanisms that drives the stochastic/deterministic balance (DINI-ANDREOTE et al., 2015; KUMAR, 2021), and priority effects (DEBRAY et al., 2021) is necessary to build a better range of knowledge on the possible environmental drivers that links the structuring of a potentially pathogenic microbiome to cattle disease.

Through the functional profiles (KEGG) predicted by Tax4Fun, we intended to address an overall perception about specific metabolic pathway classes within KO (level 2) among studied components. As a result, we observed that all the components of the soil-plant-animal continuum were enriched with sequences belonging to starch and sucrose metabolism (KO00500) which belongs to the carbohydrate metabolism pathway. Independently of the source (e.g., starch and sucrose), the high carbohydrate intake had a negative impact on periodontal health in a mouse model of naturally occurring periodontitis (MORIMOTO et al., 2019). Moreover, it was shown that starch intake tends to decrease ruminal fiber digestibility and increase the risk of dysbiosis (PETRI et al., 2020). Furthermore, it is important to highlight that the nitrogen-free extract (NFE), which represents the non-structural carbohydrates such as starches and sugars, was selected in the integrated model among the bromatological composition variables of the forage. Although the short statistical difference, NFE content was significantly higher in forage of disease-susceptible systems, which in general also had a lower balance of macro and micronutrient content in their nutritional composition, as already discussed above. Starch decomposers, such as streptomycetes, produce organic acids, CO_2, and dextrin during the decomposition process (MOREIRA; SIQUEIRA, 2006), which seems to offer the necessary substrate for the structuring of a more specialized microbial community in the context of the periodontal disease. Wang et al. (2013) detected an over-representation of genes for glycan biosynthesis in the subgingival plaques of chronic periodontal patients. Our results seem to support this evidence once the oral microbiota of diseased cattle was enrichment of 4 among 6 predicted genes within the glycan biosynthesis and metabolism group, mostly lipopolysaccharide biosynthesis (KO00540), and other glycan degradation (KO00511).

Dr. Jürgen Döbereiner and team reported that streptomycin produced by soil streptomycetes, when ingested with the forage leaf would be one of the main pathways for the development of cattle periodontitis. Based on "*in vitro*"-assays, it was confirmed that soil streptomycin significantly increased up to 10-fold the adherence of bacteria associated with periodontal disease to host gingival epithelial cells, suggesting that antibiotics from this group play

an important role in the pathogenesis of this multifactorial infectious disease (DÖBEREINER et al., 2000; GRASSMANN et al., 1997; KOPP et al., 1996). Some studies have also reported that bacterial biofilm formation is enhanced under sub-inhibitory antibiotics doses, including streptomycin, which increase their resistance to environmental stressors in comparison to their free-living/planktonic counterparts (KAPLAN, 2011; KUMAR; TING, 2016). The quantitative PCR results revealed that the proportion of *strB1*/16S rRNA gene copies of soils from disease-susceptible systems followed a significant positive correlation with the increases in exchangeable sum of bases. This is in line with the overall increases in relative abundance of ASVs belonging to *Actinobacteria* from forest to pasture soils, as previously reported in the chapter II of this thesis. In general, less fertile soils are naturally found in forests, and the more fertile ones belongs to pastures due to the effect of mineralization of forest organic matter after cutting and burning vegetation.

Finally, our results thus far provided consistent evidence to ensure that farms with a record of higher incidence of the cattle periodontal disease reflect an overall distinct microbial profile and signature in comparison to areas with a lower severity level of the disease, which is likely to culminate in a more suppressive environment itself. We suggest that abiotic factors, which permeate local edaphoclimatic conditions, and the bromatological composition of forage plants, combined with the biological response of microbiomes to disturbance events can turn the pastures more susceptible to the triggering of the pathology. Therefore, further research using both *in vitro* and multi-omic methods (e.g., transcriptomics and metagenomics) from samples of manipulated environments (e.g., pasture renewal using fertilization and liming) is encouraged to increase resolution on functional mechanisms that are modelling soil microbiomes and phyllosphere of disease-susceptible systems differently of LSL. Assessing the water retention parameters in soils from LSL and HSL systems is also suggested, considering the importance of water fluxes on chemical soil transformations (e.g., Fe redox), which is an important factor to increase levels of disturbance in soil microbiomes.

5.7 CONCLUSIONS

Considering the findings presented in this study, we revealed that disease-susceptible systems reflect the environmental disturbances through the increase in alpha and gamma diversities throughout all components of the soil-plant-animal continuum, suggesting dysbiosis not only at the level of the oral microbiota but, above all, at the ecosystem level. Moreover, it possibly results in decrease of important keystone taxa (e.g., class *Bacilli* and *Gammaproteobacteria*) related to microbial protection, which results in disruption of microbial co-occurrence network stability, favoring the predominance of specific taxa. Furthermore, a better quality in terms of soil and forage nutrients were found in systems with low severity level of the disease, with especial attention to the higher Zn and Cu content in their forage and soil, respectively. Targeted streptomycin gene also evidenced that disease-susceptible systems offer more conditions to increased streptomycin biosynthesis, which was previously reported to facilitate adherence of pathogenic bacteria to bovine epithelial cells. Collectively, although dealing with a source of uncertainty inherent of a complex dataset, our findings point out that the intensification of land-use seems to exert a different impact on disease-susceptible systems than those with lower severity level, triggering a cascade-effect that, depending on the magnitude of the arrangement between biotic and abiotic factors, sets the ideal conditions for the emergence of oral infections.

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6. CHAPTER IV

WATER FLOWS IN RECENT AND OLD PASTURES WITH DIFFERENT SEVERITY LEVELS OF CATTLE PERIODONTITIS: A CASE STUDY IN BOCA DO ACRE REGION, AMAZONIA

6.1 RESUMO

Com o aumento da pressão antrópica sobre os ecossistemas para uso e exploração, mudanças drásticas na dinâmica natural da sazonalidade da água têm sido relatadas. A conversão da floresta em pastagem é uma das questões mais delicadas do nosso tempo, principalmente nos trópicos, que conserva a maior biodiversidade existente sobre a pedosfera. As pastagens geralmente apresentam uma diminuição na porosidade total e infiltração de água em comparação com as florestas primárias e secundárias, bem como uma menor taxa de evapotranspiração efetiva, o que modifica os parâmetros do teor de água no solo. Além disso, o manejo extensivo da pecuária tende a causar degradação da estrutura do solo. A magnitude deste efeito é, entre outros, principalmente em função da classe do solo e da sua textura predominante, que responderá de forma diferente à dinâmica estacionária da chuva. Com base nesta constatação, o uso da modelagem de fluxos hídricos pode ser uma ferramenta valiosa para monitorar o impacto do uso antrópico do solo, bem como apoiar o desenvolvimento de sistemas agropecuários mais adequados para cada região, a partir de uma maior sistematização de informações sobre o balanço hídrico local. Essas ferramentas também podem ser aliadas na investigação de fatores desencadeantes de patologias que podem estar relacionados ao teor de umidade do solo. Nesse sentido, desde a década de 1960, observações de campo e dados não publicados por patologistas animais sugerem que a periodontite bovina - uma doença multifatorial e polimicrobiana com etiologia ainda não totalmente descrita - ganha maior ocorrência após as estações chuvosas, com eventos temporários de saturação do solo, no entanto esse fator não foi monitorado adequadamente até o momento. Este estudo teve como objetivo explorar o uso do software HYDRUS-1D para simular a dinâmica e fluxos da água no solo em pastagens recentes ($\leq$ 7 anos) e antigas ($\geq$ 20 anos) de fazendas na região de Boca do Acre, Amazônia Ocidental. De acordo com o histórico de ocorrência de periodontite em bovinos, as propriedades foram classificadas em alto ou baixo nível de severidade da periodontite bovina (ANS e BNS, respectivamente). De maneira geral, as pastagens do sistema BNS são mais heterogêneas entre si quanto aos valores médios de retenção de água anual do que as encontradas entre as pastagens do sistema ANS. Além disso, pequenas amplitudes internas também foram observadas entre os períodos chuvoso e seco em ambas as pastagens do sistema BNS, tanto para a profundidade de 10 quanto para 30 cm. Assim, embora limitados a este estudo de caso, especulamos que ao se considerar o manejo da rotação do gado, a maior heterogeneidade no grau de saturação do solo nas primeiras camadas do perfil do solo entre as pastagens deve fornecer condições distintas de qualidade da forragem para os animais, bem como na estruturação e atividade da comunidade microbiana do solo e da planta, com intensidades distintas de estresse ambiental, que pode ter consequências na saúde do gado.

Palavras-chave: Modelagem de fluxos hídricos. Uso antrópico da terra. Periodontite bovina. Conteúdo de água no solo. Boca do Acre.

6.2 ABSTRACT

Increasing anthropic pressure on ecosystems for use and exploitation, drastic changes in the natural dynamics of the water seasonality have been reported. The conversion of forest to pasture is one of the most sensitive issues of our time, especially in the tropics, which conserves the greatest biodiversity existing in the pedosphere. Pastures generally have a decrease in total porosity and water infiltration compared to primary and secondary forests, as well as a lower effective evapotranspiration rate, which modifies soil water content parameters. In addition, extensive livestock management tends to cause degradation of soil structure. The magnitude of this effect is, among others, primarily a function of the soil class and its predominant texture, which will respond differently to stationary rainfall dynamics that span periods of high rainfall followed by increasingly intense periods of drought. Based on this observation, the use of water flow modeling can be a valuable tool to monitor the impact of anthropogenic land-use, as well as to support the development of more suitable agricultural and livestock systems for each region, based on a greater systematization of local water balance information. These tools can also be allies in the investigation of triggering factors of health problems that may be related to soil moisture content. Along these lines, since the 1960s, field observations and unpublished data from animal pathologists suggest that the cattle periodontitis disease – a multifactorial and polymicrobial disease with an etiology still not fully described - gains greater occurrence after wet seasons, with temporary soil saturation events, however, this relationship has not been properly monitored to date. This study aimed to explore the use of HYDRUS-1D software to simulate soil water dynamics and fluxes in recent ($\leq$ 7 years) and old ($\geq$ 20 years) pastures of farms in the Boca do Acre region, Western Amazonia. According to the history of occurrence of periodontitis in cattle, the farms were classified as high or low severity level of the cattle disease (HSL and LSL, respectively). Overall, the pastures of the LSL system are more heterogeneous each other regarding the average annual water retention values than those found among the pastures of the HSL system. Furthermore, small internal amplitudes were also observed between the rainy and dry seasons in both pastures of the LSL system, both for the 10 and 30 cm depth. Thus, although limited to this case study, we speculate that when considering cattle rotation management, greater heterogeneity in the degree of soil saturation in the early layers of the soil profile between pastures should provide distinct forage quality conditions for animals, as well as in the structure and activity of the soil and plant microbial community, with distinct intensities of environmental stress, affecting the cattle health.

Keywords: Water flow modeling. Anthropic land-use. Bovine periodontitis. Soil water content. Boca do Acre.

6.3 INTRODUCTION

Forest-to-pasture conversion is known to have a huge impact on soil water flows, due to the drastic change in hydrological dynamics at different spatial scales (DOS SANTOS et al., 2018; NÓBREGA et al., 2017), especially in tropical ecosystems (NOBRE et al., 2016). It is reported that a higher available water content remains retained in pasture soils due to the metabolism of forage plants and their lower effective root system depth, differently than what is found in arboreal ecosystems (GASH; NOBRE, 1997), which, therefore, culminates in a lower effective evapotranspiration rate for this type of land-use system (HODNETT et al., 1995).

Extensive livestock production can also promote increased soil exposure to wet-dry cycles (LAURANCE et al., 2002), propitiating the formation of a waterproofing layer and soil crust on the surface due to the direct impact of raindrops and cattle trampling (LADO; BEN-HUR; SHAINBERG, 2004; MÜLLER et al., 2004), decreasing the porosity, water infiltration, and consequences like soil structure degradation (TEIXEIRA et al., 1996). The magnitude of this effect is, among others, primarily a function of soil type and its predominant textural class, which will respond differently to stationary rainfall dynamics that encompass periods of high rainfall followed by those of increasingly intense drought, as has been constantly alarmed in studies of climate change across the Amazon basin (NOBRE et al., 2016).

Faced with the drastic alterations that natural ecosystems have been suffering as a result of the advance of anthropic activities (e.g., extensive cattle raising), it is pertinent to systematize information about the impacts of land-use intensification to the detriment of the preservation of natural ecosystems and nature-based agroecosystems. To this end, the use of computational tools for the monitoring and prediction of water and energy dynamics at the Earth's surface is important due to the low cost and high accuracy of simulation models, supporting decision making on biogeophysical systems (ARTS; VAN DER WAL; ADAMS, 2015). One of the possibilities lies in the application of these tools to support the understanding of disease behavior in cultivated environments (e.g., crop and pasture systems) that have a possible relationship with water seasonality. This is supported by the fact that many reports are still limited to occasional, supplementary, unsystematic field observations, such as for those related to cattle periodontitis, once previously called "swollen face disease" or "cara-inchada dos bovinos – CIb", *in Portuguese* (DÖBEREINER et al., 2000).

Although relevant for its high impact on cattle health, with recorded cases of deaths exceeding 60% of the herd (DÖBEREINER; DUTRA; ROSA, 2004), the triggers of cattle periodontitis have not yet been fully understood (BORSANELLI et al., 2018). Considered as a multifactorial disease, in which abiotic and biotic factors interact in response to disturbances in the ecological stability of natural ecosystems, is suggested that the removal of natural vegetation cover, and/or disruption of the ecological stability of less intensive land-use systems (e.g., pasture renewal) create conditions to trigger the process that favors infections (DÖBEREINER et al., 2000). Since the 1960s, field observations and unpublished data from animal pathologists suggest that the disease gains greater occurrence after wet seasons, with temporary soil saturation events, however, this relationship has not been properly monitored to date.

Here, we aimed to use the HYDRUS-1D software to simulate the soil water dynamics and fluxes in recent (7-years-old) and old (20-years-old) pastures of farms in the Western Amazon region, near the city of Boca do Acre (Amazonas, Brazil). Among the locations accessed in this thesis, Boca do Acre is the only one with farms classified as both high and low disease severity systems, besides the similarity among the soil classes. This scenario was identified as favorable

because it allows reducing confounders arising from soil and climate heterogeneity, especially those related to soil types. Here it was hypothesized that, although under the same domain of Latosols, the differences in soil physical properties between areas of high and low disease severity give these systems distinct responses to water seasonality, which may increase the conditions for disease triggers in cattle in areas of high incidence. To tackle this objective, daily climate data from a 7-year time series were used to verify possible correlations between soil hydraulic parameters, water seasonality, and the difference in susceptibility of the pastures to the occurrence of the disease.

6.4 MATERIAL AND METHODS

6.4.1 Sampling and experimental design

See section 3.4.1.

6.4.2 Soil classification, and sampling for hydraulic parameters

Details about soil classification, see section 3.4.2. Soil physical properties bulk density, macroporosity, microporosity, and total porosity, were determined based on soil cores were taken using stainless steel rings (internal diameter of 5.7 cm, a height of 4 cm, and a volume of 102 cm3) from each soil horizon. The collected samples were wrapped into plastic for a convenient transport to the Soil Physics Laboratory at the Embrapa Amazônia Ocidental station in Manaus, Brazil, where the soil water retention curves were determined based on protocols found in Teixeira et al. (2017).

6.4.3 Meteorological parameters

The meteorological data were obtained from the official base of the National Institute of Meteorology (INMET), from the automatic weather station Boca do Acre/AM, Brazil, under number A110, contemplating the period from 2011 to 2018, with daily data periodicity (Figure 28.). To calculate evapotranspiration (ET_o), the Penman-Monteith (ALLEN et al., 1998) method was used, which includes the meteorological elements: average air temperature (°C), relative humidity (%), global solar radiation (MJ m^{-2} day^{-1}), and wind speed at a 2 meters height (m s^{-1}). In case of rainfall data gaps, data from rainfall stations belonging to the HIDROWEB/ANA (http://hidroweb.ana.gov.br) database located in the same municipality of study were used. For other meteorological elements for the calculation of ET_o, data was estimated by the NASAPOWER model (STACKHOUSE JR et al., 2018) based on the geographic coordinates of the INMET weather station previously used.

The crop potential evapotranspiration (ET_c) was calculated from the reference evapotranspiration (ET_o) and the crop coefficient (Kc). The Kc values for two stages (early: ≤ 90 days, and formation: > 90 days) of the pastures were 0.8 and 1.0, respectively (SANCHES et al., 2019).

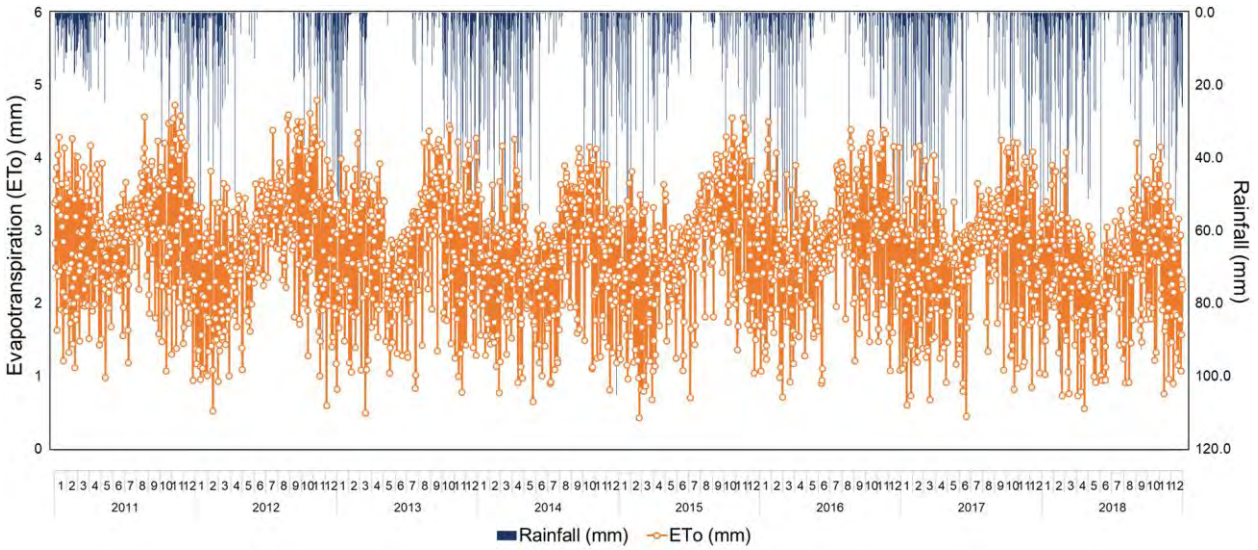

Figure 28. Temporal dynamics of daily rainfall and reference evapotranspiration (ETo) in the period of the years 2011 and 2018 for the Boca do Acre region, Amazonas, Brazil.

6.4.4 Simulation of soil water dynamics by HYDRUS-1D

HYDRUS-1D (ŠIM NEK; VAN GENUCHTEN, 2008; SIMUNEK; VAN GENUCHTEN; SEJNA, 2008) was used to simulate the daily water dynamic and fluxes along the years of 2011-2018, based on the time since conversion of the forest to the recent pasture. HYDRUS-1D uses the modified Richards equation for one-dimensional unsaturated water flow with the addition of the water extraction term by the root system (S) (RICHARDS, 1931; VAN GENUCHTEN, 1980). For modeling simplicity, hysteresis was not considered in this study.

To perform the simulation in HYDRUS-1D, besides the meteorological data, and the soil hydraulic parameters, the van Genuchten – Mualem equation parameters was used for describing the relation between volumetric water content (theta) and potential (h) displayed in Table 3. Through the RETC (Retention Curve Software) (LEIJ et al., 1992), the soil water retention curve data performed in the range of 0, -10, -30, -60, -100, -330, -1100, and -1500 kPa water potential (h) for each soil horizon. Here, h is water pressure head (tension) applied in centimeters of water. The parameter adjusted were θr: residual water content, θs: saturated water content, α: empirical parameter related to the air-entry pressure value, n: empirical parameter related to the pore size distribution width. The saturated hydraulic conductivity (Ks) was estimated based on the experience in the Amazonian soils to be around 70 cm day^{-1}.

Here, we estimate the parameters for soil horizons up to 60 cm depth, in order to cover the average effective depth of the forage root system (ALENCAR et al., 2009). The value of the pore connectivity parameter (l) was assumed to be (0.5). The coefficient of determination R^2, and root mean square error (RMSE) were used for the purpose of evaluate the model performance for each one of the hydraulic parameters obtained from the soil horizon of the soil profiles.

Table 3. Mualem-van Genuchten parameters for the hydraulic properties of soil profiles from recent and old pastures of the HSL and LSL systems in Boca do Acre, Amazonas, Brazil.

Severity level [1]	Pasture age	Horizon	Layer (cm)	θs (cm^3cm^{-3})	θr (cm^3cm^{-3})	α (cm^{-1})	n	R^2	RMSE
High (HSL)	≤ 7-year-old	A	0-7	0,2679	0,1048	0,1252	1,4539	0,9216	0,0141
		BA	7-38	0,2779	0,1045	0,0827	1,5926	0,9551	0,0120
	20-year-old	A	0-12	0,3261	0,1586	0,0732	1,5692	0,9446	0,0128
		Bw1	20-48	0,4341	0,1614	0,0642	1,8213	0,9734	0,0157
Low (LSL)	≤ 7-year-old	A	0-8	0,4487	0,2311	0,1728	1,3088	0,9715	0,0105
		Bt1	22-44	0,4896	0,2546	0,1967	1,5216	0,9918	0,0067
	30-year-old	AB	6-13	0,3035	0,0156	0,0641	1,2667	0,9705	0,0136
		BA	13-36	0,2428	0,0955	0,0215	2,9955	0,9600	0,0124

[1] Severity level of cattle periodontitis based on clinical assessment and farm history. θr: residual water content, θs: saturated water content, α: empirical parameter related to the air-entry pressure value, n: empirical parameter related to the pore size distribution width; R^2: coefficient of determination, and RMSE: root mean square error.

According to Feddes et al. (1978), four matric potentials (here used as root zone pressure head) determine the extraction of water by the crop root system, where ‘h0’ refers to the point from which the roots begin to extract water from the soil; at ‘h_{opt}’ the plants extract water from the soil at the highest possible rate; at ‘h2’ the plants can no longer extract water at the maximum rate, which is the limit matrix potential, where above this (‘h3’) water absorption is prevented.

Graphical analyses were performed using the ‘aqp’ v.1.32 (BEAUDETTE; ROUDIER; O’GEEN, 2013), and ‘ggplot2’ v.3.3.5 (VILLANUEVA; CHEN, 2019) packages and their dependencies in the R environment.

The meteorological data in Julian days, the data for input into HYDRUS-1D, and parameterization for the simulation models are available in the ANEXO D.

6.5 RESULTS AND DISCUSSION

Overall, distinct characteristics were observed with respect to textural attributes, bulk density, and soil porosity in soil profiles among the evaluated pastures. The recent pastures (≤ 7 years old) of the farm characterized with low severity level (LSL) of cattle periodontitis showed 3 times higher clay content along the soil profile compared to the old pastures (≥ 20 years old), which have 2.5 times higher fine sand content (g kg^{-1}) (Figure 29).

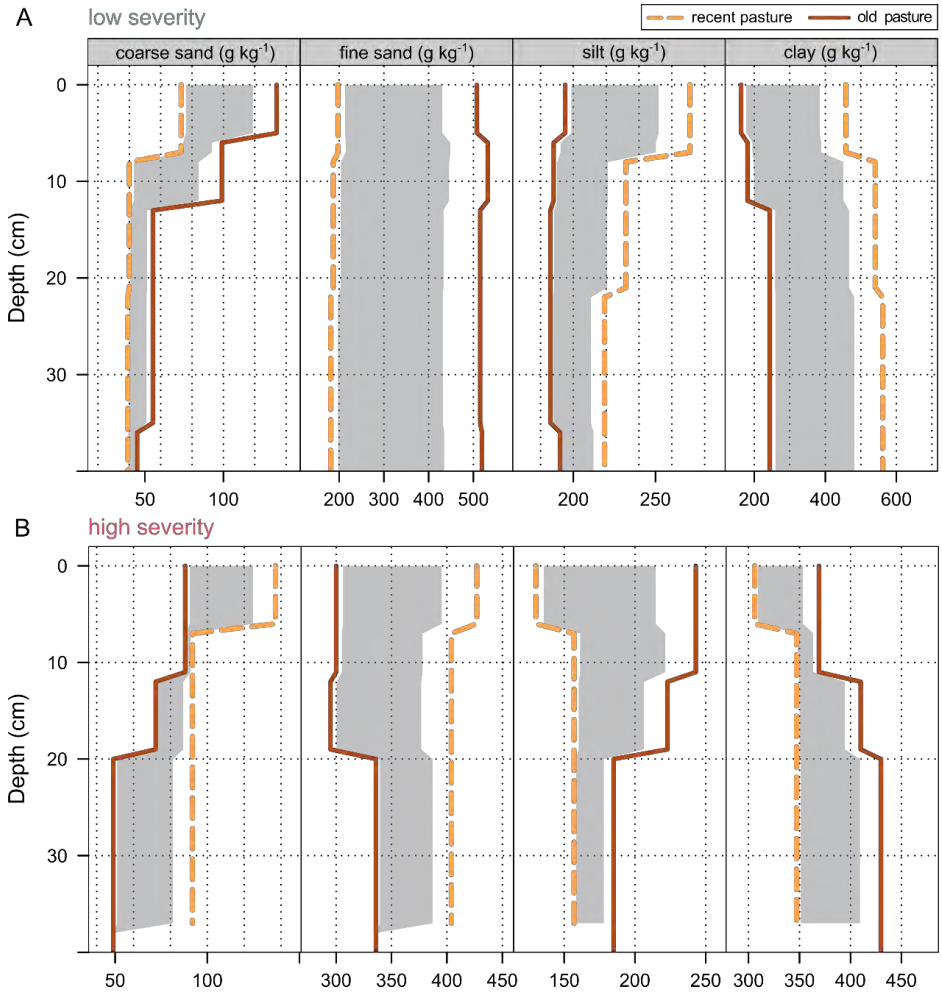

Figure 29. Vertical distribution of soil physical parameters in recent and old pastures of high (HSL) and low (LSL) incidence bovine periodontitis systems in the Boca do Acre region, Amazonas, Brazil. Median bounded by 25th and 75th percentiles.

The farm with higher disease severity level (HSL), in turn, showed contrary patterns to those observed for the LSL system. The old pasture has a higher clay and silt content, and a lower sand content (coarse and fine), although the differences are not as great as those found for the pastures in the HSL system. Consequently, the above-mentioned characteristics imply differences in soil density and porosity conditions between the evaluated pasture systems (Figure 30).

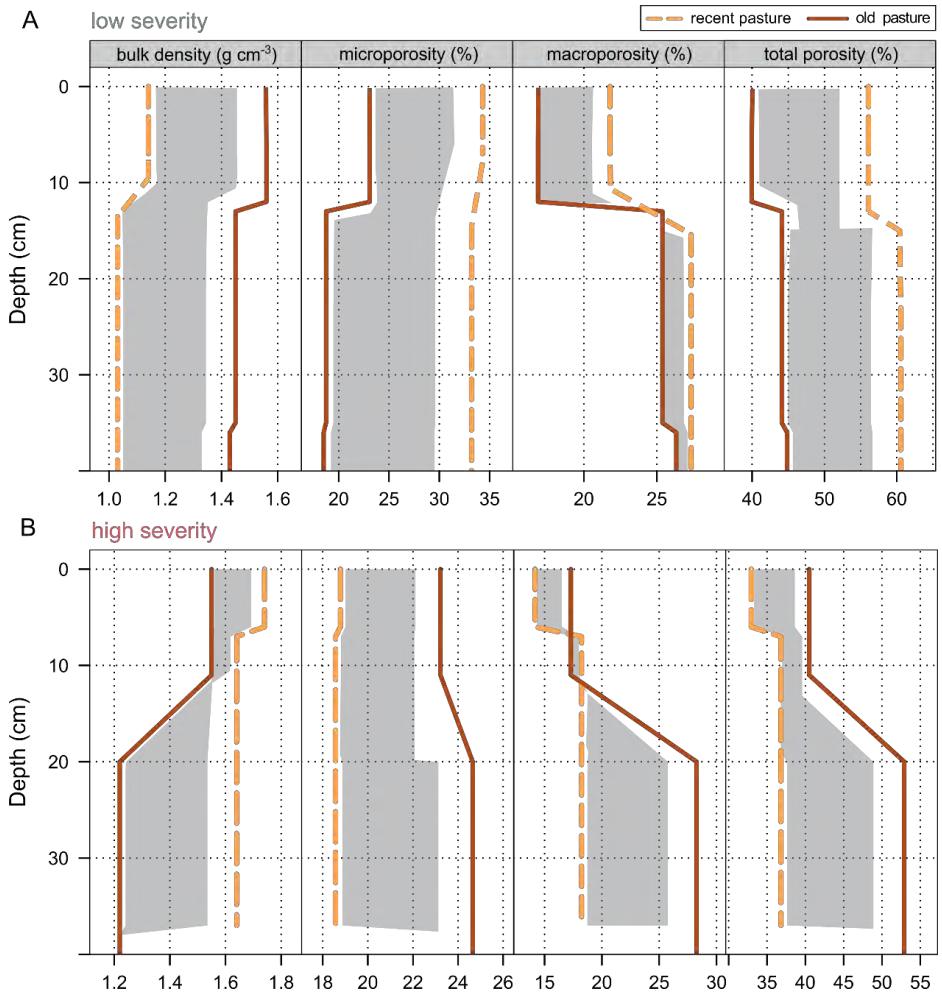

Figure 30. Vertical distribution of soil hydraulic parameters in recent and old pastures of high (HSL) and low (LSL) incidence bovine periodontitis systems in the Boca do Acre region, Amazonas, Brazil. Median bounded by 25th and 75th percentiles.

Contrary to our expectations, the old pastures of the HSL system evidenced a higher percentage of total pore volume (+30%) compared to the recent pastures, and consequently lower soil bulk density (-25%), especially below 20 cm of the soil profile. Although the variations are not as high compared to the pastures of the LSL system, they apparently show that differences in pasture age are not enough to imprint relevant effects on the soil profile of this system.

The greatest differences observed in the total porosity of the soil profiles between recent pastures was addressed to the microporosity (pores < 50 µm), which is responsible for large amount of the soil water storage (PORTUGAL et al., 2008). The recent pasture of the LSL system presents around 32% of microporosity throughout the soil profile compared to 18% found in the HSL system. All the pastures evidenced higher bulk density in de first 0-15 cm than the bottom layers, moreover, only the recent pasture of LSL system showed bulk density bellow 1.4 g cm^{-3}, whose value is pointed out as the critical value for root system development for most soils when in field capacity (SOUZA; CARNEIRO; PAULINO, 2005), although this does not have to be taken as a rule.

Nevertheless, isolating the effects of pasture management from pedogenetic factors is not trivial. Even though the evaluated soils belong to the same soil order of Ferralsols (*Latossolos*), they have different taxonomic classifications at the more specific categorical levels, being Pisoplintic and Plintic Ferralsols (*Latossolo Amarelo Distrófico petroplíntico*, and *plintossólico*, respectively) in the recent and old pastures of the HSL system, respectively. Both LSL pastures were classified as Xanthic Ferralsol (*Latossolo Amarelo*), although they differed in the predominance of the textural class (i.e., clayey/very fine clayey, and loamy for recent and old pastures, respectively). However, it is necessary to carry out periodic monitoring in the soil environment to measure transformations in soil properties to generate more reliable datasets. This inverse pattern observed to soil attributes in pastures of the same age highlights that, despite the similar period since the conversion of the forest into pasture, the magnitude of the response to land-use management seems to be more determined by the pedogenetic factors, especially those related to the weathering of primary minerals. The main textural characteristic of the soil turns it more or less susceptible to land-use intensification impacts on soil bulk density and porosity, which directly impact variations in water retention and flow in the soil profile, as will be seen below.

During the period of evaluated (2011-2018), soil water storage (SWS) in the 100 cm soil profile showed that among recent pastures, the LSL system had a higher SWS over the timeseries compared to the HSL, with an annual average of 353 ± 40 mm, where 318 ± 30 mm occurring along the dry season (July-September) and 371 ± 40 mm for the wettest months (October-May) (Figure 31). On the other hand, the HSL system had an annual average of 170 ± 30.11 mm, with 144 ± 24 mm for the dry season, and 182.6 ± 26 mm for the rainy season. Interestingly, this pattern was reversed when observed the SWS for old pastures. The HSL system, in turn, showed higher values in comparison to LSL, with an annual average of 243.6 ± 41.4 cm, with 210.4 ± 31.9 mm in the driest months, and 259.8 ± 35.5 mm in the rainiest ones. The LSL system showed an annual average of 122.9 ± 21.6 mm, being 106.0 ± 15.5 mm between the months of Jun-Sep, and 131.2 ± 19.2 mm between Oct-May. Considering the rate for annual mean SWS between recent and old pastures, we observe that the LSL system has an average value of 230.5 ± 20.3 mm of SWS apart between the pastures, while the HSL system has 73.6 ± 9.2 mm, which indicates that SWS is found under similar conditions in the recent and old pastures of the HSL system.

Despite the higher temporal average of SWS in the recent pasture of the LSL system, the estimated cumulative actual water uptake by the forage was higher in the HSL system, suggesting a more efficient water use dynamic than in recent pasture of the LSL system. It was recorded from the second year of prediction onwards, with a value 1.4 times higher (= 1123 mm) at the last measurement. This behavior was not observed among old pastures whose values was similar throughout the evaluation (HSL = 4000 mm; LSL = 3700 mm in the last measurement). In general, actual water uptake tends to be greater in old pasture than recent ones, mostly in the LSL system where the widest difference (1.5 times higher = 1230 mm) was reported.

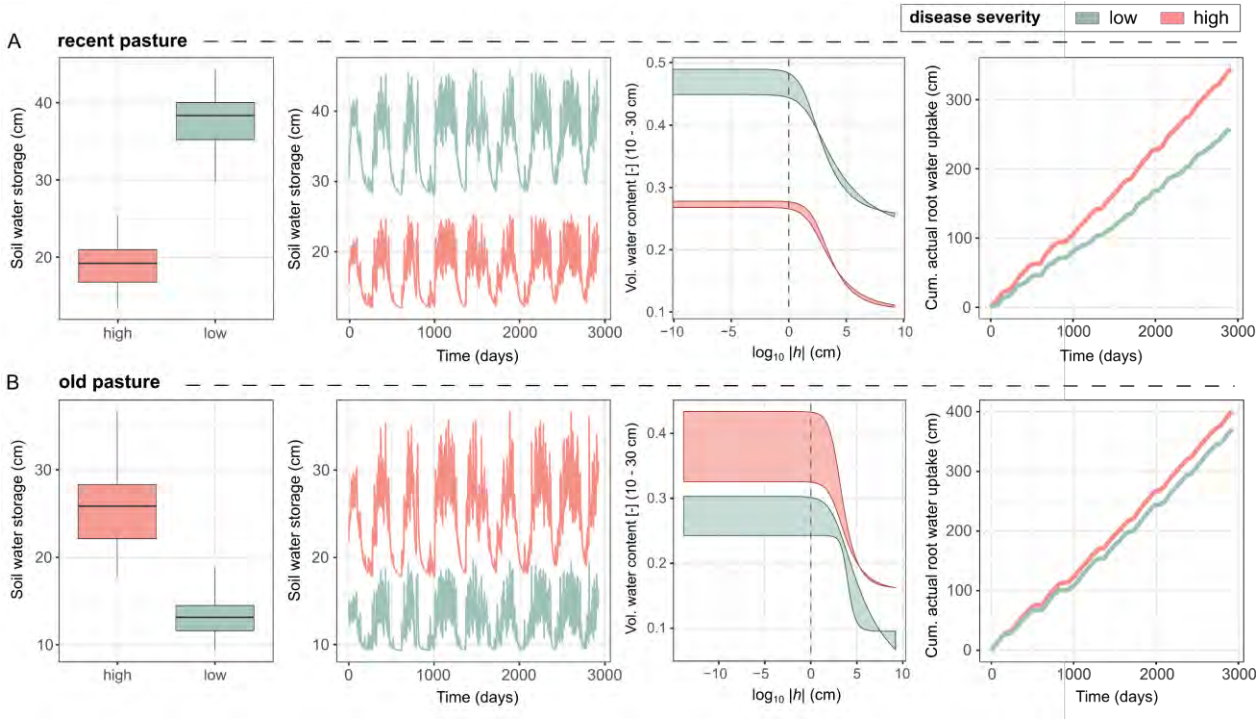

Figure 31. Soil water storage (SWS), volume of water content (cm^3 cm^{-3}), and cumulative actual root water uptake along the years of 2011-2018 in recent and old pastures of high (HSL) and low (LSL) incidence bovine periodontitis systems in the Boca do Acre region, Amazonas, Brazil.

The attraction between aggregates during the drying process can be explained by the forces of attraction related to the capillary film between soil particles (FLURY; ARAMRAK, 2017). In saturated material, the space between the aggregates is filled with water and the attraction force is zero, while with the loss of water occurs the meniscus formation with negative radius, which increases the capillary action (BRINI et al., 2017). The magnitude of the impacts of wetting-drying cycles is highly related to the physical, chemical, and mineralogical properties of each soil type whose influence affects aggregate stability and bulk density. Such factors determine the nutrient availability and microbial dynamics of these environments (BARNARD; OSBORNE; FIRESTONE, 2013). In addition, this process can cause the dissolution and deposition of soil compounds that act as cementing agents (e.g., silica, carbonates, and oxides) (BACHMANN et al., 2021).

Using the parameters of volumetric water content (θ; cm^3 cm^{-3}) and saturated soil water content (θs; cm^3 cm^{-3}), it was possible to obtain the predicted percentage of void volume filled with water (i.e., degree of saturation, henceforth DS) for the 10 cm and 30 cm soil layers over the 8-year simulation time period (Figura 32). The recent pastures in the HSL system were highly affected by the annual precipitation dynamics in both soil layers, evidencing a seasonal variation of about 50% DS throughout the year during the evaluated time series (wet season, max = ~ 90%; dry season, min = ~ 40%). Still in the HSL system, maximum values of up to 80% DS at 10 and 30 cm were observed in all rainy season in either recent or old pastures. Minimum values of about 40% were recorded in the dry seasons in recent pastures, as well as in the 30 cm depth of the soil profile of old pastures, which recorded a ~ 50% minimum DS in the 10 cm layer for the same

period of the year. This is probably due to the aforementioned fact that the recent pastures have a finer sand content than the old pastures, which in turn have higher total porosity and a clay content of about 30 cm layer. Given this information, it can be defined that the pastures (recent and old) of the LSL system are more heterogeneous with each other than those of the HSL in terms of DS amplitudes for the same soil depth. At both 10 cm and 30 cm, the DS percentage in the old pastures dropped by about 20% when the pastures are compared considering the same period of the year, while the values for the HSL system pastures remained similar at both depths when compared within the same period of the year.

Based on experimental data from Franzluebbers (1999), Yan et al. (2016) simulated scenarios of variation in organic carbon availability (measured by CO_2 efflux) and pore scale saturation degrees to measure heterotrophic soil respiration rates. Values around 75% of DS determine the highest respiration rates by the heterotrophic microbiota, with an accentuated reduction due to limitations in the diffusion of O_2 when DS values increase. For the authors, organic carbon bioavailability and oxygen supply are the two most important factors that affect the effective soil respiration rate as a function of moisture content.

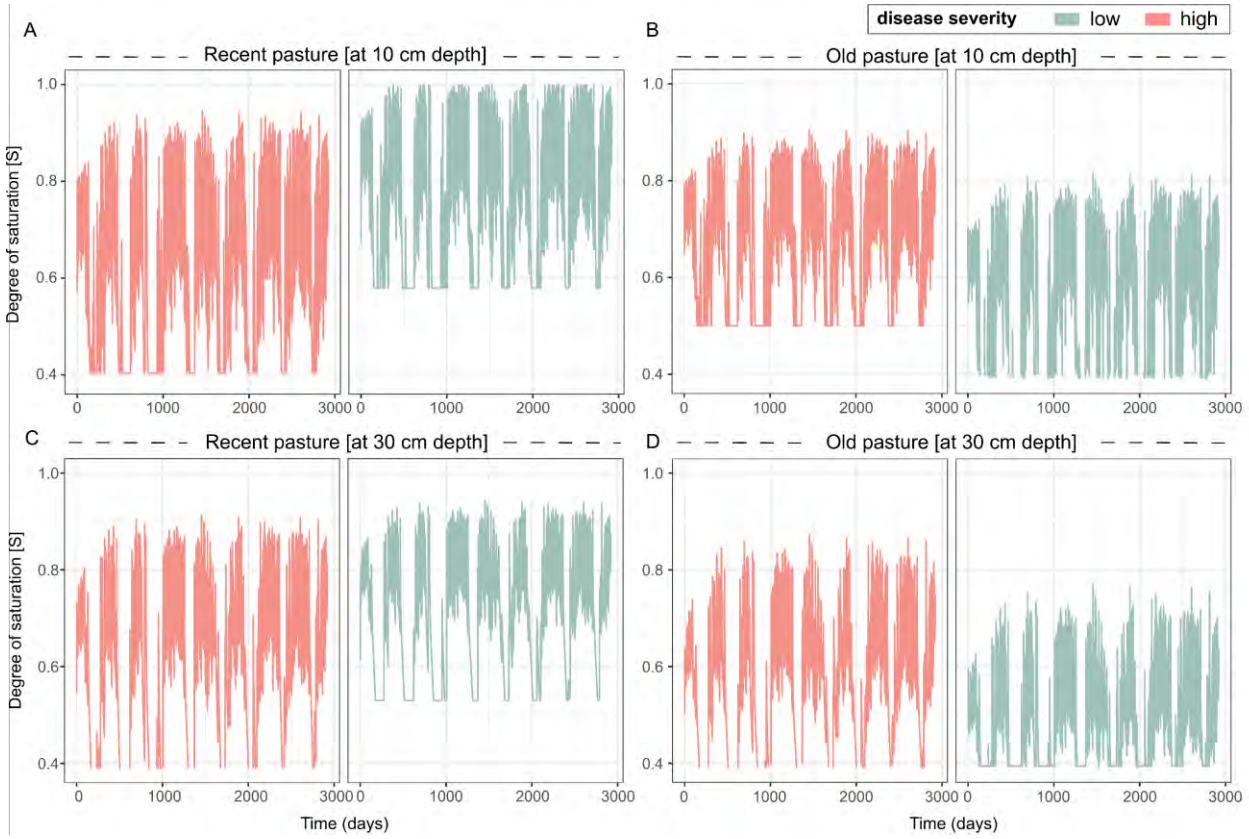

Figure 32. Daily measurement of the degree of soil saturation (DS%) at 10 cm and 30 cm depth along the years of 2011-2018 in recent and old pastures of high (HSL) and low (LSL) incidence bovine periodontitis systems in the Boca do Acre region, Amazonas, Brazil.

Oxygen is necessary for aerobic respiration and soluble organic substrates are the energy sources for heterotrophic microorganisms. For this, the optimum water content is generally close to field capacity, where the macropores are mostly filled with air, facilitating the diffusion of O_2,

and the micropores become filled with water, facilitating the diffusion of these soluble substrates (LINN; DORAN, 1984). In a scenario of high oxygen consumption in the vadose zone, especially in the first centimeters of the soil surface, there is the establishment of anaerobic microorganisms that start to promote chemical transformations such as the reduction of nitrate to nitrite, and later transformed and released into the atmosphere in the form of N_2 and N_2O. Furthermore, there is a reduction of Mn and Fe oxides, and sulfate, as the redox potential decreases. Conversely, when soils are very dry (e.g., pressure head lower than –10 kPa), small differences in volumetric water content result in large differences in pressure head. Davidson et al. (2000) reported that respiration rates generally decreased with decreasing water content in pasture and forest soils in the Eastern Amazonia. It was also speculated that during the driest season the water stress develops more quickly in shallower soils and is affected not only by the soil surface pressure head, but also by the total amount of water contained in the soil profile. Another issue that should also be considered refers to the depth of biological activity in soils, as this can vary with climate, soil type and vegetation.

Considering the importance of water holding capacity in the root zone in determining the overall rates of water fluxes in the soil layers by plant transpiration, we applied the default Feddes parameters (FEDDES; KOWALIK; ZARADNY, 1978) for pasture system available on HYDRUS-1D Database upon the interpolated temporal data from the root zone pressure head (RZPH; Figure 32) distributed in decreasing root volume over 60 cm in depth. It was observed that the magnitude of the effects presented for the DS (Figure 32) converged in relevant periods of water stress in all evaluated pastures. For recent pastures, the LSL system showed greater mean amplitude throughout the year, with mean RZPH values smaller than h_{opt} with periods close to -10 cm in the rainy season ($CI_{max} < h0$), and mean values between $h2_L$ and *h3* along the Jun-Sep months, with maximum peaks below the wilting point ($CI_{min} >$ - 8000 cm; *h3*) in September. The old pasture of the HSL system did not vary much from the mean distribution pattern of the RZPH seen for the recent pasture of the same system, except for the peaks of CI_{max} close to *h0* in the rainy season. A smaller amplitude of the RZPH was observed for the old pasture of the LSL system, according to the previous results, with maximum mean values below h_{opt} and minimum above *h3*, which suggests that this pasture is exposed to a lower frequency of events of water stress, either by excess or by very negative matric potential where the access to water by plant forage is hampered.

According to Ripley et al. (2010), C4 grasses are metabolically more sensitive to drought than C3 species, although C4 species have a buffering mechanism against early drought. The decrease of C4 photosynthesis under water stress is primarily due to stomatal closure (GHANNOUM, 2009), and under water deficit, the CO_2 concentration in the leaves may decrease because of decreased stomatal conductance which may drive the plant to increase the photorespiration rate (HABERMANN et al., 2019). This information is relevant to infer about the response to water stress of different forage plants used in the evaluated pastures, moreover, in scenarios of drastic variations in soil water supply, especially considering the high average rainfall in the Western Amazonia (> 2200 mm), varying in months with an average rainfall above 300 mm and others below 50 mm.

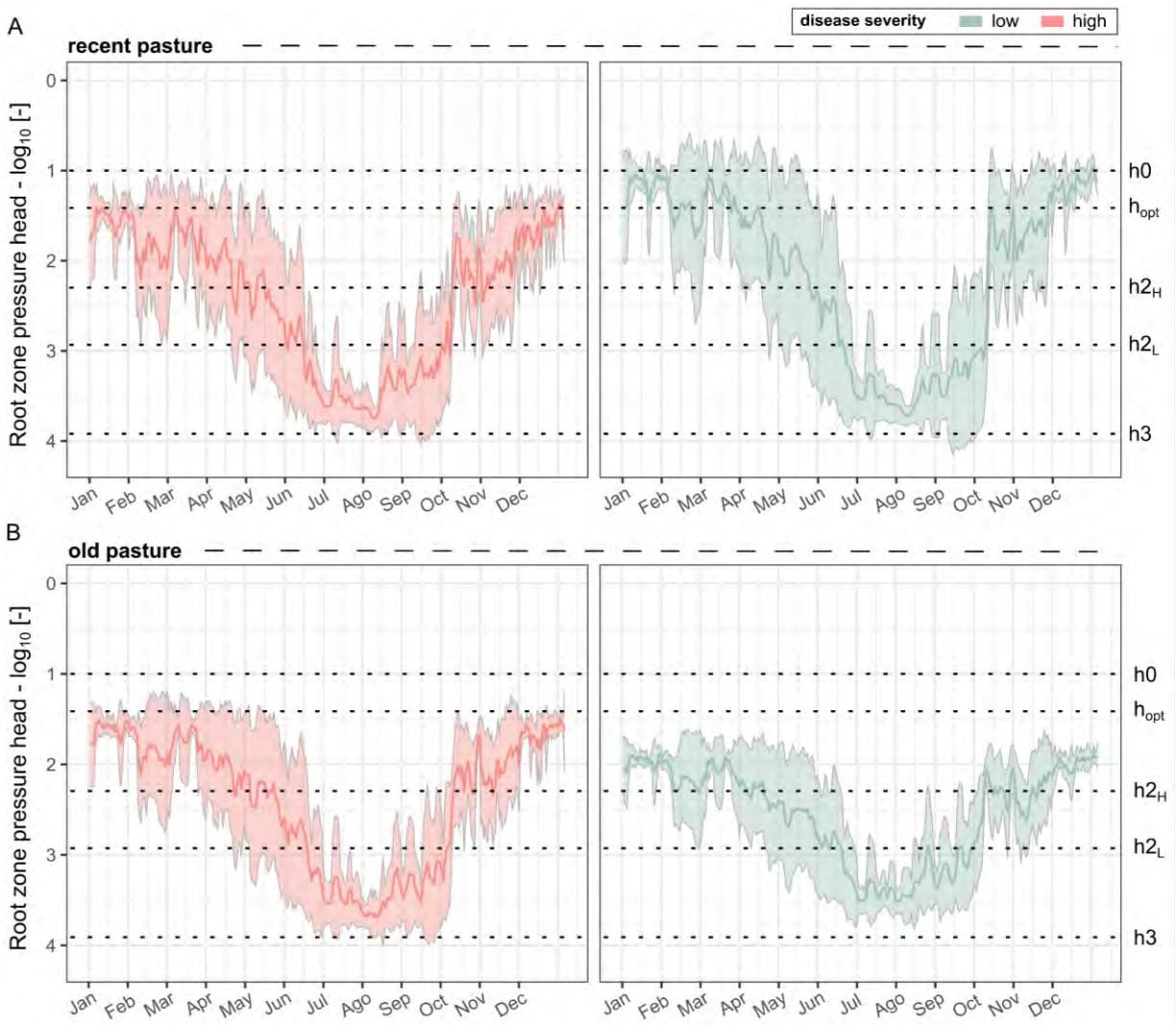

Figure 33. Daily temporal variation of root zone pressure head (RZPH) in interpolated data from the years 2011-2018 in recent and old pastures of high (HSL) and low (LSL) incidence bovine periodontitis systems in the Boca do Acre region, Amazonas, Brazil. Feddes parameters are overlapped on the y-axis: (oxygen deficiency (h0) = -10 cm, between hopt = -25 cm, and h2H = -200 cm or h2L = -800 cm, the water uptake is optimal, and wilting point pressure head (h3) = -8000 cm) was adopted considering the suggested values for pasture systems, available in HYDRUS-1D. The strong line represents the mean, and maximum and minimum lines refer to the confidence intervals (CI).

The LSL system is formed with the forages Panicum maximum cv. Mombaça and Urochloa humidicula in recent pasture, and U. humidicola + U. brizantha cv. Marandu in the old pasture, being P. maximum cv. Mombaça + U. humidicula characterized as tolerant to periods of high soil moisture and U. brizantha cv. Marandu as suitable for periods of low water availability (VÉRAS et al., 2020). On the other hand, the recent and old pastures of the HSL system have U. brizantha cv. Xaraés + cv. MG5, and U. brizantha cv. Marandu + U. humidicola, respectively.

6.5.1 Conjectures on soil climate as a conditioning factor for the incidence of cattle periodontitis

Soil microorganisms live in an ecosystem that is dominated by solid particles, which have a large surface area. The surface area assigned to the bacterial population is 10^{10} cells g^{-1} and is considered small compared to the total specific surface area of soils with different textures (CHENU; STOTZKY, 2001). According to Baveye and Darnault (2017), in the porous media, other microorganisms are inevitably present and can affect not only the competition and evolution of bacteria directly, but also the hydrodynamics of the pore space. Still on Baveye and Darnault (2017), is pointed out that hydrodynamics can have a considerable effect on the dynamics of protozoan predators, predatory bacteria, or viral particles (phages), all of which are ubiquitous in natural porous media and directly influence the bacterial populations. The growth of fungal hyphae can also transport bacteria (and archaea) from one portion of the pore space to another, as well as partially clog pores (FALCONER et al., 2015).

It is predicted that dry periods result in declines in microbial function whose activity is linked to available water content (CURIEL YUSTE et al., 2007). This assumption has been shown in studies that correlates drought to lasting impacts on the soil microbiome in grasslands, as vegetation shifts to more drought-tolerant plant species and subsequently selects different root-associated soil microbes (DE VRIES et al., 2018). In situations of low water availability, the microbiota starts to use survival strategies that invokes osmotic adjustments that require the availability of organic substrate (WARREN, 2014; YAN et al., 2016). Depending on the limitation of this resource, below a critical level in degree of saturation ($S < 12\%$ was reported by Yan et al., 2016) microorganisms enter a state of dormancy or sporulation, as the case of the phylum *Actinobacteria*, as an effective strategy to resist against drought. Using NGS analysis on 16S rRNA gene and transcriptomics, Barnard et al. (2013) revealed that bacterial community structure can be completely insensitive to extreme dry-rewet events even though bacterial activity can be strongly affected. Other studies using PLFA (Gordon et al., 2008) or DNA-based techniques (FIERER; SCHIMEL; HOLDEN, 2003; KAISERMANN et al., 2015) also reported the stability of bacteria to variations in soil moisture. To the best of our knowledge, there is no research to date that has evaluated the fluctuation of soil microbiota as a function of rainfall seasonality in tropical environments, especially those that have performed actual measurements of hydraulic parameters to establish further quantitative relationships of this scenario. Moreover, the temporal variation of the microbiota in different compartments of forage plants is even less known or unknown, being a knowledge gap to be filled.

The investigations carried out by Dr. Dobereiner and collaborators since the 1960's to unveil the processes that trigger the outbreaks of bovine periodontitis in Brazil ("cara-inchada dos bovinos - CIb" *in Portuguese*), as well as the international studies reported for "broken-mouth" by (WEST; SPENCE, 2000) do not consistently provide evidence that define a pattern of occurrence of the disease or associate modification in the clinical picture of the disease according to the dry and rainy periods. Here, we sought to expand the knowledge on the subject by modeling water fluxes in recent and old pastures of high and low disease incidence systems (HSL and LSL, respectively) using an 8-year historical rainfall series, aiming to verify the existence of patterns that can differentiate the systems based on parameters related to soil water retention. Overall, the pastures of the LSL system are more heterogeneous each other regarding the average annual water retention values than those found among the pastures of the HSL system. Furthermore, small internal amplitudes were also observed between the rainy and dry seasons in both pastures of the LSL

system, both for the 10 and 30 cm depth. Thus, we speculate that when considering livestock rotation management, greater heterogeneity in the degree of soil saturation in the early layers of the soil profile between pastures should provide distinct forage quality conditions for animals, as well as in the structure and activity of the soil and plant microbial community, with distinct intensities of environmental stress. As already mentioned, HSL systems subject animals to constant exposure to soils with DS above 90% in the wet season, and values below 50% in the dry season, which can mean large oscillation in stress to the soil environment and consequently to the plants, affecting the cattle health.

The impacts of abiotic stress (e.g., drought or oxygen starvation) on forage plant metabolism is extensively studied (BARRETO et al., 2020; BORJAS-VENTURA et al., 2019; SANDERSON; STAIR; HUSSEY, 1997; VICIEDO et al., 2019), and it is for example reported that water deficit can stimulate root growth, acting as an active sink for sugars, and that water stress and warming can increase lignin content, and decrease leaf starch and crude protein content, decreasing forage digestibility (HABERMANN et al., 2019). Furthermore, under conditions of proximity to soil water saturation, there are possible increases in leaf sugar contents (in line with one of the findings presented in chapter 3 of this thesis), indicating that carbohydrate supply is not a limiting factor in anaerobic metabolism in *Brachiaria* species (RAM, 2000).

Finally, considering that bovine periodontitis is a multifactorial disease (DÖBEREINER et al., 2000), it is plausible to indicate that the present study was limited to provide signals regarding the heterogeneity of response to the water dynamics of pastures of farms reported with a history of high and low incidence of cattle periodontitis, respectively, even though sharing the same pedological and climatological domain. Thus, the inclusion of the soil chemical properties along the soil profile, as well as a more refined monitoring of the soil-plant-animal continuum microbiome associated with water parameters is encouraged to robustly dissect the possible relationship between the effect of the rainfall seasonality and the transformations in the soil climate with the onset of the periodontitis disease in cattle.

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7. GENERAL CONCLUSIONS

Regarding the first central axis of this thesis ("*Effect of land-use change on biodiversity of the edaphic environment*"), it can be concluded that the magnitude of transformations of the soil environment as a function of land-use change is largely determined by pedogenetic factors. Chemical properties are rapidly altered when forest is converted to pasture, especially the soil sum of bases, which was the most sensitive for all study sites. The prokaryotic metacommunity also responds to transformations in the soil environment, with drastic rearrangements in the composition and abundance of prokaryotic communities, depending on the conditions under which the pasture soil is exposed. In terms of microbial biodiversity, the components of the forest floor host a high beta diversity, thus ensuring spatial heterogeneity. However, the effects on ecological functions were not explored in this thesis.

For the second central axis ("*Relationship between land-use change, local abiotic factors, and the increased susceptibility of pastures to trigger periodontitis in cattle* "), it was consistently evidenced that high severity level (HSL) cattle periodontitis systems have different environmental vectors than those found for low severity level (LSL), apparently leading to greater environmental disturbance in these systems. This was concluded based on the difference in core microbiota structure, higher alpha and beta diversity across all components of the soil-plant-animal continuum in HSL systems. This is possibly reflecting the greater modularity observed both in the global co-occurrence networks (generated by integrating the abundance matrices of soil, forage, and subgingival biofilm DNA sequences) and individually, for soil, forage, and cattle microbiota. The lower importance of keystone taxa such as *Bacillus spp.* and *Pseudomonas spp.*, related to microbial protection, and higher for sequences of the class *Bacterodia* in forage and cattle, in addition to the higher relative abundance of streptomycin biosynthesis in the soil-forage-animal continuum, also support the conclusion about the disruption of the ecological stability. The lower Zn and Cu content in the soil, and the lower forage quality in HSL systems may signal factors to be observed in future experimental studies.

Overall, the collected results allow the conclusion that land-use intensification exert a different impact on HSL systems than LSL, triggering a cascading-effect that, depending on the magnitude of the arrangement between biotic and abiotic factors, establishes ideal conditions for the onset of oral infections. Finally, it is possible to consider that this study raises information that supports new scientific hypotheses, both for the study of microbial biodiversity and for a better understanding of the etiology of periodontitis in cattle. The monitoring of key abiotic factors, as well as the key microbial groups detected, should be explored in field monitoring and further genomic analysis.

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9. ATTACHMENTS

9.1 Chapter I Supplements

Table 4. Subset of important soil variables by principal component analysis (PCA) and their factor loadings for PC1 and PC2.

Selected soil variables	BUJ		BAC1		BAC2		MAN1		MAN2	
	PC1	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**	**PC1**	**PC2**
fine sand	**-0.29**	0.14	**-0.26**	0.10	**-0.26**	0.10	-0.12	-0.20	-0.20	0.16
silt	**0.23**	**-0.20**	**0.22**	0.05	**0.22**	0.05	0.21	-0.17	0.01	**-0.39**
clay	**0.30**	-0.00	**0.26**	-0.15	**0.26**	-0.15	-0.06	-0.20	0.19	-0.18
CDW	**0.28**	0.07	**0.24**	-0.15	**0.24**	-0.15	0.19	-0.09	0.21	-0.16
pH_{H2O}	0.16	**0.26**	0.04	**0.42**	0.04	**0.42**	**0.30**	-0.08	**0.30**	-0.02
$Ca^{+}Mg$	**0.30**	0.12	**0.25**	0.17	**0.25**	0.16	**0.29**	-0.04	**0.29**	-0.02
SB	**0.29**	0.13	**0.25**	0.16	0.19	**-0.31**	**0.29**	-0.05	**0.28**	-0.02
Al_3^{+}	-0.07	**0.36**	0.19	**-0.31**	**0.26**	-0.07	**-0.28**	-0.00	**-0.26**	-0.17
H^{+}	**0.21**	**-0.30**	**0.26**	-0.15	**0.25**	-0.20	**-0.25**	**-0.25**	-0.17	**-0.33**
H+Al	0.19	**-0.33**	**0.25**	-0.20	**0.26**	-0.15	**-0.28**	-0.18	-0.20	**-0.30**
T-CEC	**0.31**	-0.00	**0.26**	-0.15	0.10	0.40	-0.15	**-0.36**	-0.12	**-0.35**
BS%	**0.21**	**0.31**	0.10	**0.40**	-0.03	-0.44	**0.29**	-0.02	**0.28**	0.00
m%	-0.18	**-0.29**	-0.03	**-0.44**	0.17	0.20	**-0.28**	-0.11	**-0.29**	0.04
P	0.01	**-0.33**	0.17	0.20	**0.25**	0.03	**0.28**	-0.02	0.10	-0.16
C	**0.29**	-0.07	**0.25**	0.03	**0.27**	-0.02	-0.06	**-0.42**	-0.07	**-0.40**
N	**0.30**	-0.08	**0.27**	-0.02	0.19	0.09	0.00	**-0.42**	-0.00	**-0.41**
K	0.10	**0.24**	0.19	0.09	**0.25**	-0.00	0.18	**-0.31**	**0.26**	-0.02
Cu	0.14	**-0.31**	**0.25**	-0.00	**0.25**	-0.00	0.00	-0.13	**0.25**	-0.12
Mn	-0.00	-0.09	0.16	**0.34**	0.16	**0.34**	**0.26**	**-0.22**	**0.27**	-0.06
Zn	-0.00	**0.31**	**0.22**	-0.02	**0.22**	-0.02	-0.10	**-0.28**	**0.23**	-0.11

(*) bold values represents those variables which were important in the PCA based on the contribution criterion. The contribution of a variable for a given principal component (PC) was obtained by the ratio of the squared component loading of the variable by the eigenvalue associated with the PC, following Abdi and Williams (2010). All those variables with a contribution larger than the cutoff of 5% (i.e., 100 x [1/20 variables]) were considered as important to the PC.

Table 5. Description and characterization of the soil profiles across Brazilian Western Amazonian regions and their land uses.

Horizon	Depth	Granulometric composition				pH		Sorptive complex			base saturation	Al saturation	C-Org
Symbol	cm	CS	FS	silt	clay	H_2O	KCl	sum of bases	Al^{3+}	CEC eff.	V%	m%	g kg^{-1}
		g kg^{-1}						$cmol_c$ kg^{-1}			%		
P1 - *Latossolo Amarelo Distrófico petroplíntico,* loamy/clayey texture, moderate A horizon (Pisoplinthic Ferralsol) - Boca do Acre /AM - **Forest**													
A	0-15	53	436	204	307	3.9	3.7	0.3	2.2	9.4	3	88	16,2
BA	15-37	51	387	152	410	4.6	3.9	0.1	1.8	5.8	2	95	6,2
Bw1cf	37-76	27	382	139	452	4.6	3.9	0.1	1.7	5.5	2	94	3,9
Bw2cf	73-120+	23	316	169	492	4.8	3.9	0.1	1.9	5.3	2	95	3,6
P2 - *Latossolo Amarelo Distrófico petroplíntico plintossólico,* loamy/gravelly clayey texture, moderate A horizon (Pisoplinthic Ferralsol) - Boca do Acre /AM – **Recent pasture**													
A	0-7	137	427	130	306	4.7	3.9	1.3	1.1	8.2	16	46	14,2
BA	7-38	92	404	157	347	4.8	3.9	0.8	1.2	6.2	13	60	7,1
Bw1cf	38-70	78	378	135	409	4.7	3.9	0.8	1.4	6.5	12	64	4,8
Bw2cf	70-93	67	390	135	408	4.7	3.8	0.4	1.7	5.5	7	81	3,3
Bw3cf	93-120+	72	345	154	429	4.8	3.9	0.2	1.6	5.3	4	89	2,9
P3 - *Latossolo Amarelo Distrófico plintossólico,* clayey texture, moderate A horizon (Plinthic Ferralsol) - Boca do Acre /AM – **Old pasture**													
A	0-12	88	300	243	369	4.5	3.8	0.8	2.2	9.5	8	73	18,6
AB	12-20	72	295	223	410	4.5	3.9	0.3	2.0	7.8	4	87	10,3
Bw1	20-48	49	336	185	430	4.7	3.9	0.1	2.2	6.4	2	96	5,0
Bw2	48-85	45	334	170	451	4.9	3.9	0.1	1.8	5.3	2	95	4,0
Bwf	85-120+	58	442	192	308	5.2	4.0	0.1	1.3	4.5	2	93	2,5
P4 - *Latossolo Amarelo Distrófico argissólico,* loamy texture, moderate A horizon (Xanthic Ferralsol) - Boca do Acre/AM - **Forest**													
A	0-8	176	634	69	121	4.0	3.4	0.7	1.5	7.7	9	68	11,4
BA	8-20	140	608	69	183	4.2	3.6	0.2	2.3	6.9	3	92	6,1
Bw1	20-35	128	617	32	223	4.3	3.6	0.1	2.6	6.3	2	96	2,8
Bw2	35-60	124	609	44	223	4.5	3.8	0.1	2.5	5.5	2	96	3,0
Bw3	60-100	128	613	36	223	4.5	3.8	0.1	2.5	4.9	2	96	1,7
Bw4	100-120+	121	605	32	242	4.5	3.8	0.1	2.5	4.7	2	96	1,2
P5 - *Latossolo Amarelo Alumínico típico,* clayey/very fine clayey texture, moderate A horizon (Xanthic Ferralsol) - Boca do Acre/AM – **Recent pasture**													
A	0-8	73	198	271	458	4.1	3.5	1.4	4.1	17.9	8	75	24,3
BA	8-22	40	187	232	541	4.2	3.5	0.3	6.3	13.3	2	95	9,0
Bw1	22-44	39	181	219	561	4.3	3.6	0.3	7.0	13.7	2	96	7,6
Bw2	44-78	23	150	180	647	4.4	3.6	0.1	7.8	13.5	1	99	6,2
Bw3	78-120+	34	170	145	651	4.5	3.6	0.1	7.6	13.3	1	99	3,8

To be continued…

Table 5 – Continuation

Horizon	Depth	Granulometric composition				pH		Sorptive complex			base saturation	Al saturation	C-Org
Symbol	cm	CS	FS	silt	clay	H_2O	KCl	sum of bases	Al^{3+}	CEC eff.	V%	m%	g kg^{-1}
		g kg^{-1}						$cmol_c$ kg^{-1}			%		
P6 - *Latossolo Amarelo Distrófico argissólico,* loamy texture, moderate A horizon (Xanthic Ferralsol) - Boca do Acre/AM – **Old pasture**													
Ap	0-6	134	508	195	163	4.9	3.9	1.0	1.0	8.0	12	50	15,1
AB	6-13	99	532	188	181	4.8	3.8	0.4	1.6	6.5	6	80	8,0
BA	13-36	55	515	186	244	4.6	3.7	0.1	2.6	6.1	2	96	3,4
Bw1	36-64	45	519	192	244	4.7	3.7	0.1	2.8	6.0	2	97	2,6
Bw2	64-120+	41	526	189	244	4.6	3.7	0.1	2.6	5.5	2	96	1,9
P7 - *Argissolo Amarelo Alumínico plintossólico,* loamy/clayey texture, moderate A horizon (Plinthic Acrisol) - Bujari/AC - **Forest**													
A	0-12	61	438	296	205	5.2	4.3	3.6	0.1	8.0	45	3	10.50
AB	12-30	47	427	260	266	4.9	3.9	0.9	1.6	5.8	16	64	4.10
BA	30-48	59	393	241	307	4.4	3.8	0.7	3.0	6.3	11	81	2.70
Bt	48-57	30	351	234	385	4.8	3.7	0.6	4.0	7.5	8	87	2.60
Btf1	57-75	12	303	208	477	4.7	3.7	0.6	4.6	9.0	7	88	2.80
Btf2	75-120+	13	259	185	543	4.8	3.7	0.7	6.4	10.2	7	90	2.30
P8 - *Luvissolo Háplico Pálico gleissólico,* loamy/clayey texture, moderate A horizon (Stagnic Luvisol) - Bujari/AC – **Recent pasture**													
A	0-5	87	71	551	291	5.5	4.5	10.5	0.1	16.3	64	1	23,0
AB	5-20	77	77	472	374	5.6	4.2	9.8	0.3	13.9	70	3	7,2
Bt1	20-35	40	59	396	505	5.1	3.8	10.9	2.7	17.8	61	20	4,1
Bt2	35-65	36	51	404	509	5.0	3.7	10.5	4.9	19.7	53	32	2,5
Btg1	65-86	42	47	445	466	5.0	3.7	11.1	6.1	21.0	53	35	2,4
Btg2	86-120+	47	62	465	426	5.1	3.7	14.4	6.1	23.9	60	30	1,7
P9 - *Plintossolo Argilúvico Alumínico gleissólico,* loamy/clayey/very fine clayey texture, moderate A horizon (Stagnic Plinthosol) - Bujari/AC – **Old pasture**													
A	0-5	139	164	407	290	5.2	4.2	7.4	0.2	14.5	51	3	27,7
AB1	5-23	130	222	360	288	5.3	4.1	5.9	0.2	9.8	60	3	8,3
AB2	23-33	129	234	350	287	5.5	4.1	5.4	0.3	8.8	61	5	3,9
2Btgf1	33-45	110	185	226	479	5.0	3.7	6.5	3.2	13.1	50	33	4,6
2Btgf2	45-76	94	176	185	545	5.0	3.7	4.9	5.5	14.6	34	53	3,7
2Btgf3	76-120+	93	165	127	615	4.8	3.7	3.4	10.1	17.7	19	75	4,0

To be continued…

Table 5 – Continuation

Horizon	Depth	Granulometric composition				pH		Sorptive complex			base saturation	Al saturation	C-Org
Symbol	cm	CS	FS	silt	clay	H_2O	KCl	sum of bases	Al^{3+}	CEC eff.	V%	m%	g kg^{-1}
		g kg^{-1}						$cmol_c$ kg^{-1}			%		
P10 - *Argissolo Amarelo Distrófico latossólico,* sandy/loamy texture, moderate A horizon (Xanthic Acrisol) - Manicoré/AM - **Forest**													
A	0-8	387	446	86	81	3.8	3.5	0.2	1.2	6.0	3	86	10,5
AB	8-19	306	506	107	81	3.6	3.7	0.2	1.3	4.3	5	87	7,3
BA	19-35	266	508	85	141	4.0	3.9	0.1	1.0	3.7	3	91	3,5
Bt1	35-57	236	498	105	161	4.3	4.1	0.1	0.8	2.6	4	89	2,3
Bt2	57-85	232	482	104	182	4.5	4.2	0.1	0.7	2.4	4	87	2,0
Bt3	85-150+	228	498	23	251	4.7	4.2	0.1	0.6	1.9	5	86	0,8
P11 - *Latossolo Amarelo Distrófico argissólico,* loamy texture, moderate A horizon (Xanthic Ferralsol) - Manicoré /AM – **Recent pasture**													
Ap	0-6	418	361	120	101	5.4	4.8	4.0	0.1	8.9	45	2	19,0
AB	6-22	308	419	91	182	4.8	4.1	1.1	0.4	5.2	21	27	8,3
BA	22-38	267	419	91	223	4.8	4.2	0.4	0.5	3.7	11	56	5,2
Bw1	38--68	245	401	172	182	4.8	4.3	0.1	0.5	3.1	3	83	3,3
Bw2	68-102	204	370	143	283	4.8	4.3	0.2	0.5	2.5	8	71	2,0
Bw3	102-150+	221	385	70	324	5.0	4.4	0.2	0.3	1.5	13	60	1,5
P12 - *Latossolo Amarelo Distrófico argissólico,* sandy/loamy texture, moderate A horizon (Haplic Ferralsol) - Manicoré /AM - **Forest**													
A1	5-13	775	97	27	101	3.7	3.6	0.1	1.4	5.5	2	93	9,6
A2	13-28	619	137	62	182	4.0	4.0	0.1	1.5	6.2	2	94	12,0
A3	28-52	506	172	79	243	4.4	4.3	0.1	1.1	4.9	2	92	8,4
AB	52-68	454	182	80	284	4.6	4.3	0.1	0.9	4.1	2	90	5,3
BA	68-89	485	166	86	263	4.7	4.3	0.1	0.6	2.2	5	86	2,3
Bw1	89-127	485	168	44	303	4.8	4.2	0.1	0.6	1.6	6	86	1,3
Bw2	127-150+	471	162	64	303	4.8	4.2	0.1	0.6	1.6	6	86	1,3
P13 - *Latossolo Amarelo Distrófico argissólico,* loamy/clayey texture, moderate A horizon (Xanthic Ferralsol) - Manicoré /AM – **Recent pasture**													
Ap	0-9	636	57	64	243	3.9	3.8	0.5	1.5	7.2	7	75	12,1
AB	9-22	505	77	73	345	4.0	3.9	0.1	1.2	5.3	2	92	8,2
BA	22-47	404	83	107	406	4.4	4.1	0.1	0.9	3.9	3	90	5,7
Bw1	47-72	416	75	83	426	4.6	4.2	0.1	0.5	2.4	4	83	3,1
Bw2	72-100	382	75	76	467	4.8	4.2	0.1	0.6	2.1	5	86	1,9
Bw3	100-150+	369	79	86	466	4.6	4.3	0.1	0.5	1.6	6	83	2,0

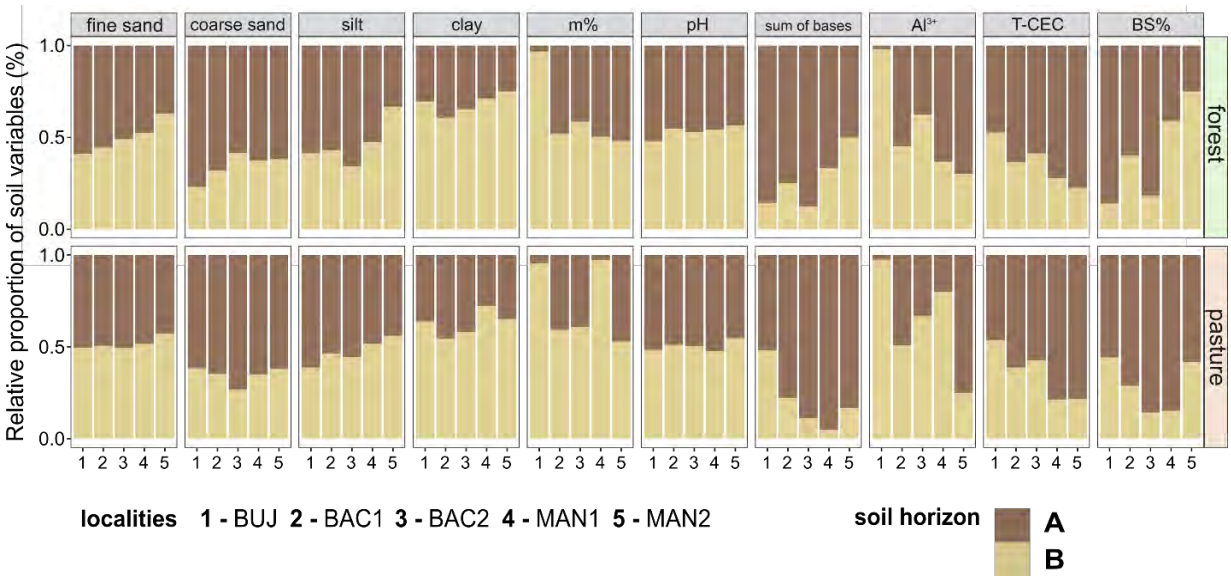

Figure 34. Relative proportion of soil variables between soil horizons A and B in different Amazon regions and land uses.

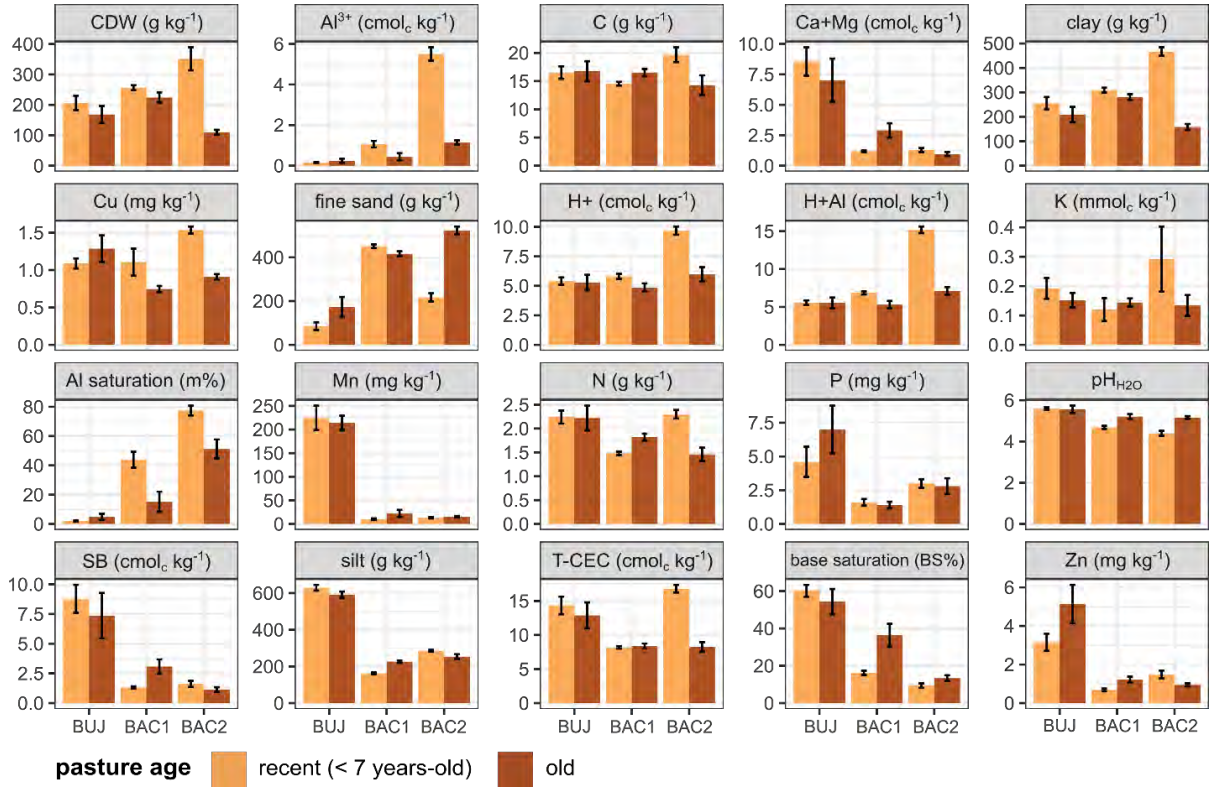

Figure 35. Variations between pasture ages for selected soil variables. Soil variables were extracted as important in the principal component analysis among different study locations in Western Amazonia. Error bars indicate the ± standard error (SE) (n = 5). BUJ: Bujari/ state of Acre, BAC1 and BAC2: Boca do Acre/ state of Amazonas.

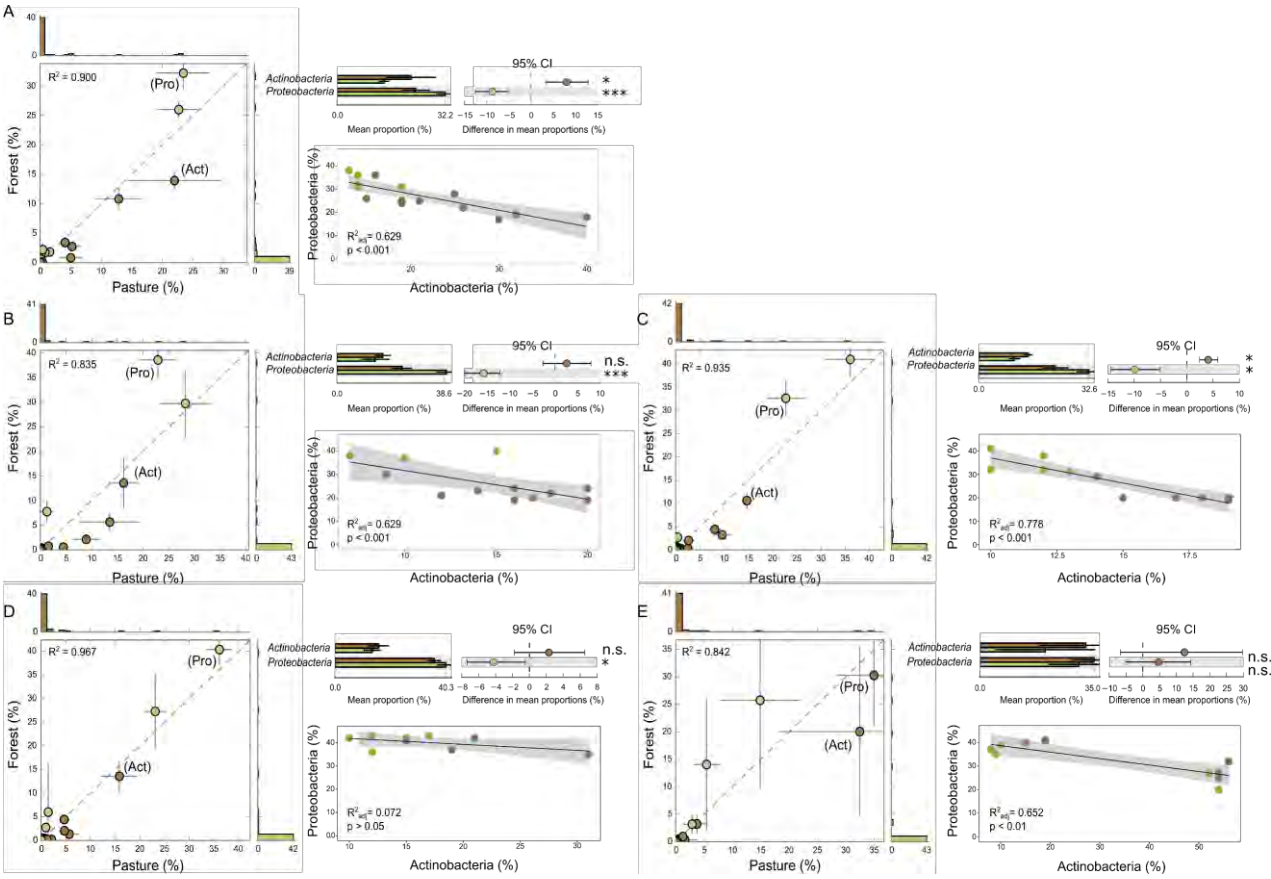

Figure 36. Testing the potential of the phyla *Actinobacteria* and *Proteobacteria* to represent land-use change due to their ecological characteristics. The graphic mosaic includes a scatter plot on the left, where each point represents a prokaryote phylum, and its position indicates the differential abundance between forests and pastures; an extended error bar in the upper right corner elucidating the statistical difference between Actinobacteria (Act) and Proteobacteria (Pro) between land uses (Welch t-test $p < 0.05$ with Benjamini-Hochberg false discovery rate); and a scatterplot in the bottom right corner with the Spearman rho correlation between the relative abundances of the tested phyla as indicators; A) BUJ; B) BAC1; C) BAC2; D) MAN1; and E) MAN2.

9.2 Chapter II Supplements

9.2.1 Soil variable selection

Principal component analysis (PCA) was used to compare samples originating from the studied soil fertility gradient across the sites. The data was standardized using *(xi – mean(x))/sd(x),* where *mean(x)* is the mean of x values, and *sd(x)* is the standard deviation (SD). The total contribution of a given variable on principal component axes was estimated. For example, the observed contributions of a variable on two principal components, say PC1 and PC2, was calculated with the formula: *[(C1 * Eig1) + (C2 * Eig2)] / (Eig1 + Eig2)*, where C1 and C2 are the contributions of the variable to PC1 and PC2, and Eig1 and Eig2 the eigenvalues of PC1 and PC2, respectively. The expected average contribution of a variable to PC1 and PC2 is: *[(number*

*of variables * Eig1) + (number of variables * Eig2)] / (Eig1 + Eig2).* In this study, the expected value was 1/length(variables) = 1/20 = 5%. In our results, variables with a contribution larger than this cutoff were considered as important in contributing to associated components. The visualization of the PCA was created with the *PCA()* function of the 'FactoMiner' R package v.2.3 (HUSSON; LÊ; PAGÈS, 2017).

9.2.2 Selected soil variables and correspondence in the structuring of soil microbial communities

Variables were selected based on their explained variance of the PCA. Next, a Constrained Analysis of Principal coordinates (CAP) was performed with these variables and a matrix of Bray-Curtis distances (capscale (formula = distance ~ silt + pH + BS% + Al saturation + Ca+Mg, data = data)) using the *ordinate()* function of 'phyloseq' v.1.30.0 (MCMURDIE; HOLMES, 2013). All selected soil variables revealed a significant relationship with the structure of the prokaryotic metacommunity (i.e., assemblage of communities) and estimated significance of correlation between soil variables and observed differences between samples.

9.2.3 Diversity partitioning analysis

Diversity profiles (i.e., alpha (α), beta (β), and gamma (γ) diversities) were calculated according to Hill numbers (for more details see Chao et al. (2014). The ASV richness, the exponential of Shannon's entropy, and the Simpson index were calculated by giving the values 0, 1, and 2 for the Hill parameter q, respectively. When q = 0, the Hill index is sensitive to ASV abundance. All individuals are equally weighted when q = 1, and the index is insensitive to the dominant species when q = 2. This approach was used to evaluate the contribution of each compartment and the overall forest floor (i.e., litter, root layer, and bulk soil) for each diversity scale. The package 'entropart' v.1.6.1 (MARCON; HÉRAULT, 2015) was used. Specifically, the *DivProfile()* function was used to calculate alpha, beta and gamma diversities. The *MergeMC()* function was used to aggregate the data present in each of the compartments into a single object, which made it possible to extract the diversity measures for the forest floor.

Finally, to test our hypothesis that the alpha, beta, and gamma diversities of the forest compartments combined are higher than in that of the pasture soil, we used the Kruskal-Wallis test to detect statistical differences. Prokaryotic diversity was compared between the forest and pasture soils as well as between the forest floor and pasture soil. The comparison was based on the overall effective numbers (i.e., Hill's q 0, 1 and 2). All p-values were corrected for false discovery rate (Benjamini-Hochberg FDR correction).

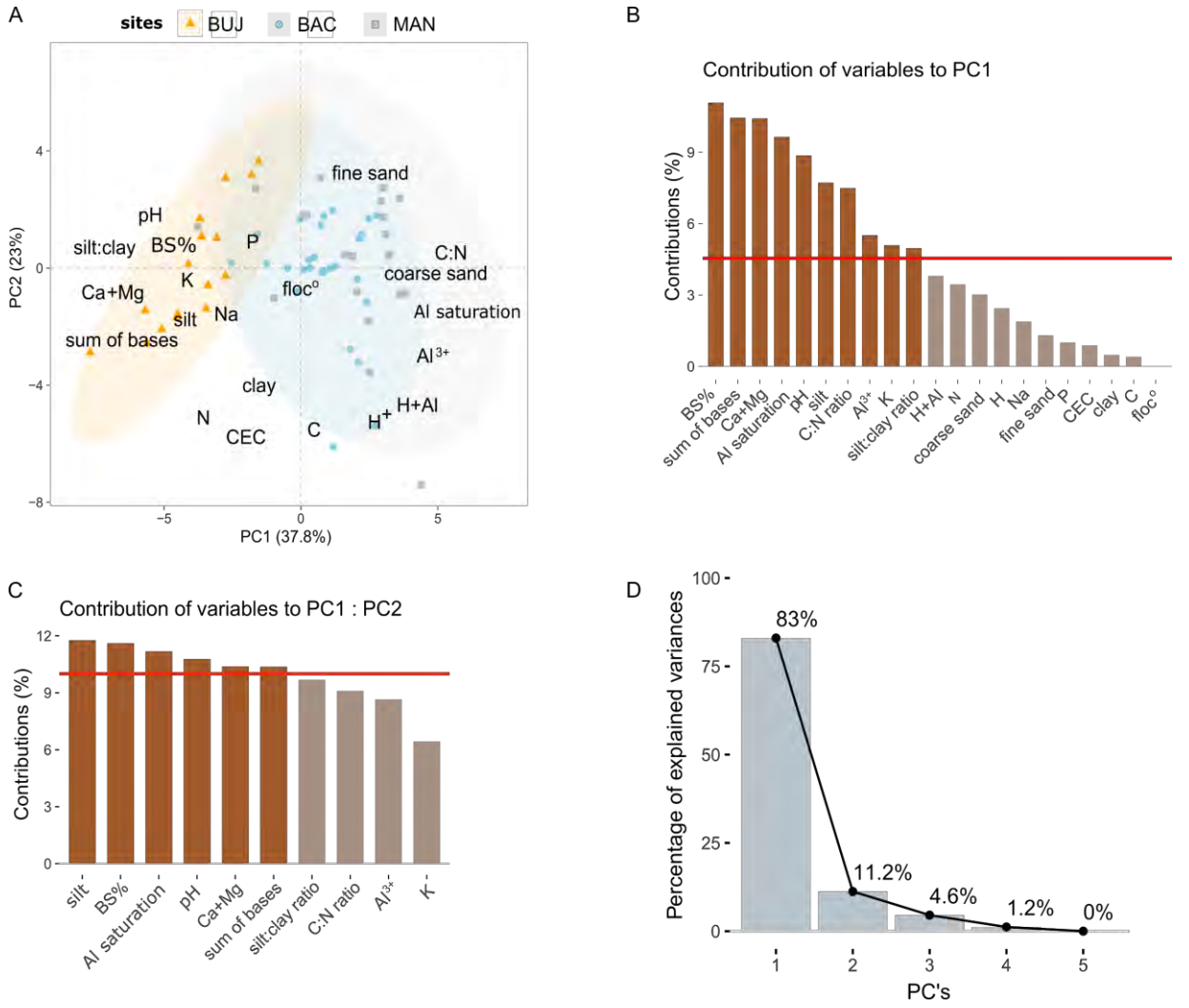

Figure 37. Soil variable selection. A principal component analysis (PCA) of soil chemical and physical variables was calculated A), and the variables that contributed to PC1 above a given threshold (red line) B) were selected and used for a new PCA. They went through a further selection, now based on their loadings on both PC1 and PC2 C). The remaining variables explained a large amount of the explained variances (%), showing the first axis as the most important to detect the gradient of fertility among the study sites D).

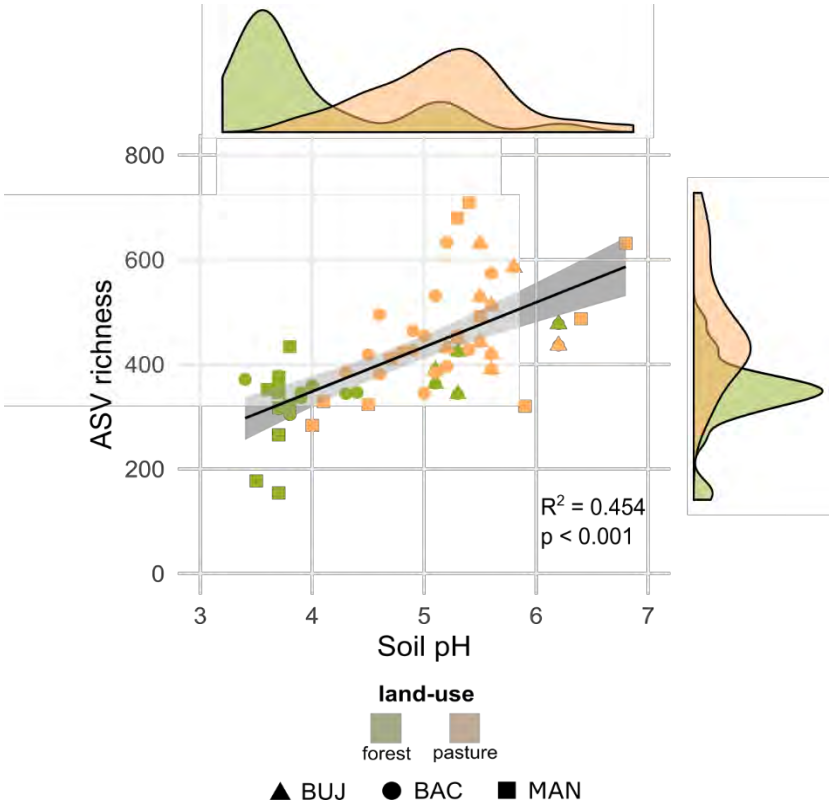

Figure 38. Soil pH effects on the ASV richness in forest-to-pasture conversion sites of the Western Brazilian Amazonia. Correlation between ASV richness and soil pH of each evaluated site in both land uses. The fitted values for each model are represented by the black line and their standard errors are indicated by the shaded area.

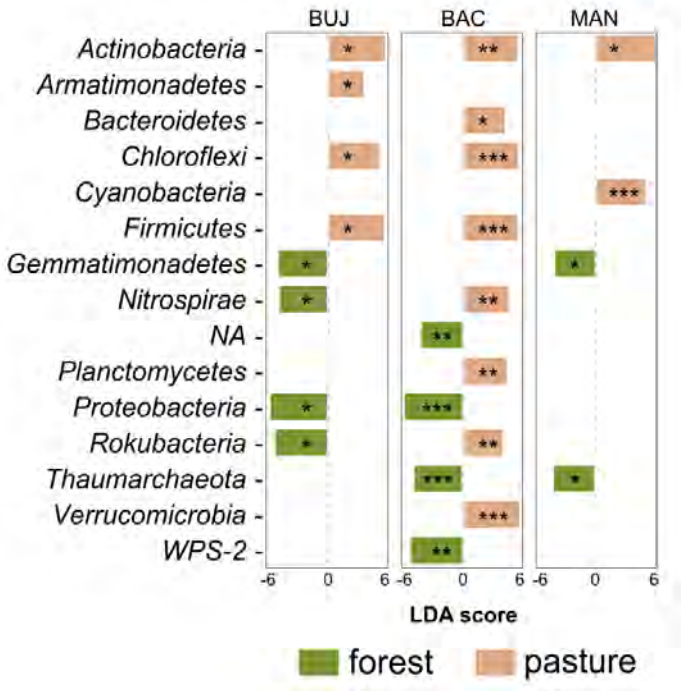

Figure 39. Soil prokaryotes with significantly different abundances between land uses. LEfSe multivariate analysis (false discovery rate (FDR) adjusted p-value < 0.05, LDA > 2.0) between land uses in Bujari (BUJ), Boca do Acre (BAC) and Manicoré (MAN).

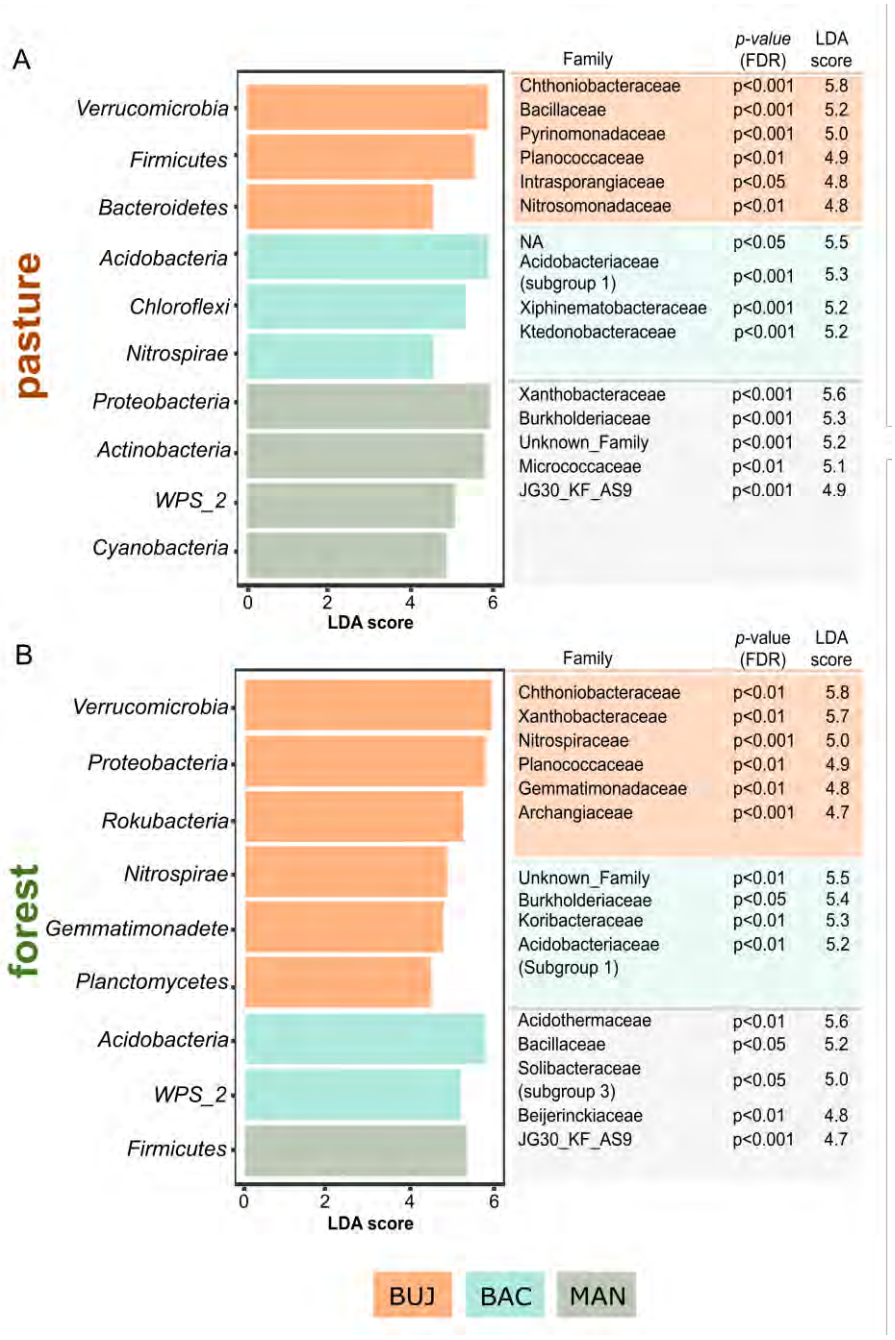

Figure 40. Soil prokaryotes with significantly different abundances within land uses across study sites. LefSe multivariate analysis (false discovery rate (FDR) adjusted p-value < 0.05, LDA > 2.0). Comparisons of the prokaryotic communities (phylum and family levels) between sites in the A) pasture and B) forest.

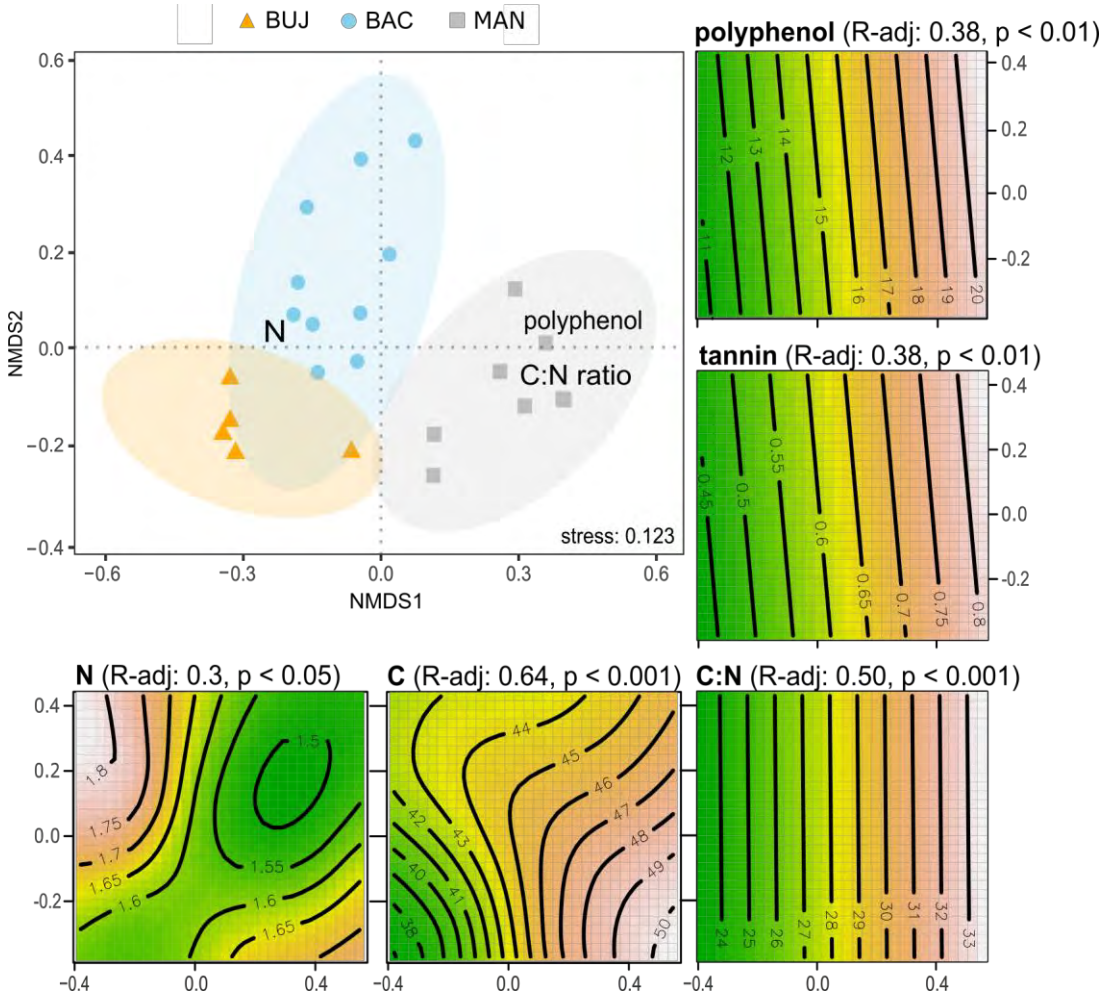

Figure 41. Prokaryotic community structure in the litter varies with litter chemistry in Brazilian Western Amazonian forests. Nonmetric multidimensional scaling (NMDS) based on a Bray-Curtis dissimilarity matrix showing variation in community structure in function of the litter chemical variables and tested by generalized additive models (GAM).

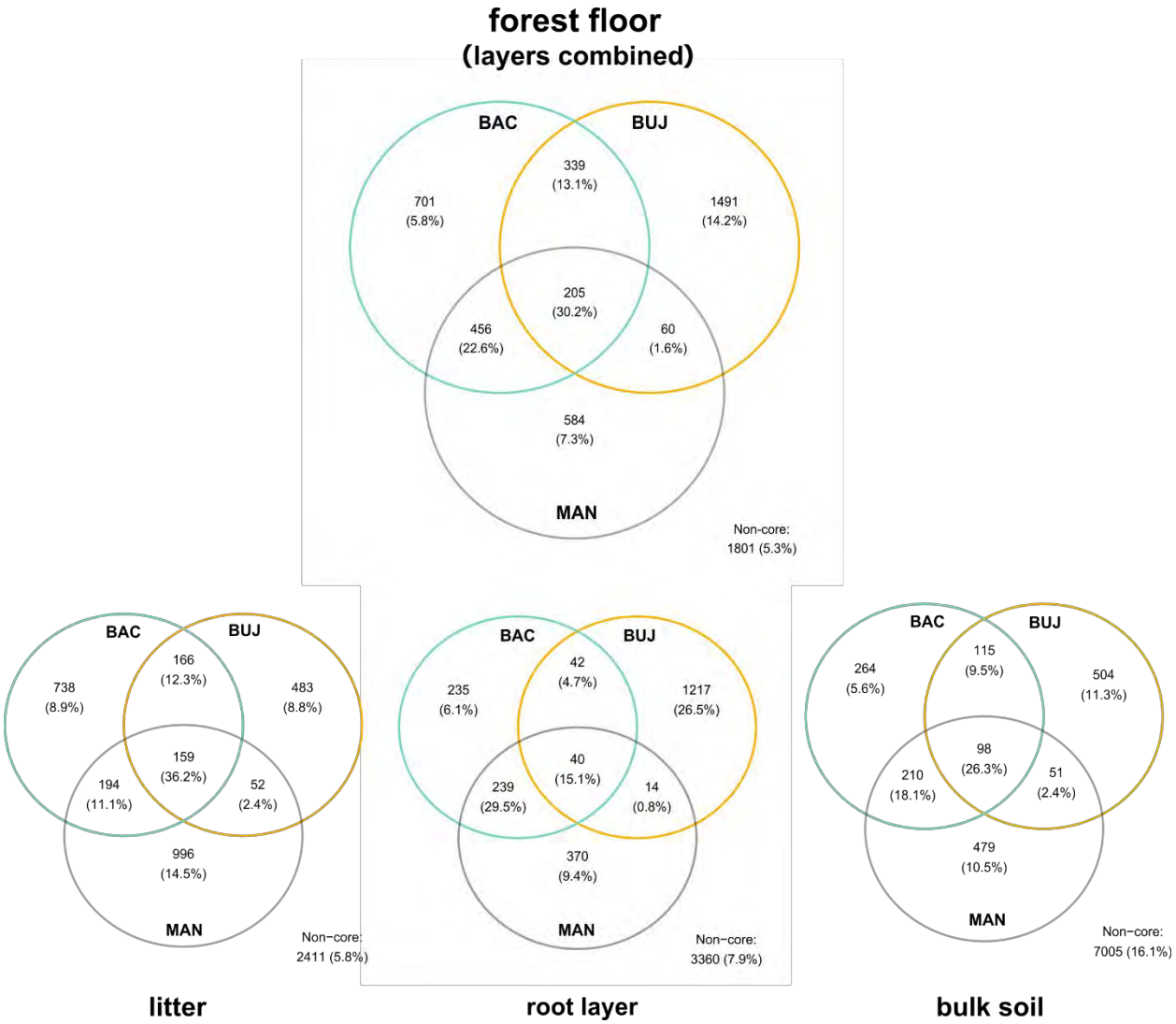

Figure 42. ASVs shared between regions and forest floor compartments. Venn diagram for each compartment of the forest floor, showing the most frequent (observed in at least 10% of the samples) and abundant (relative abundance in each sample greater than 0.01%) ASVs.

Table 6. Soil and landscape features, pedological descriptions, and geographic coordinates of the regions and land-use systems selected in the Brazilian Western Amazonia.

Profile	Brazilian System of Soil Classification (SiBCS)	Soil classification (WRB/ FAO)	County/ State	UTM coordinates (datum WGS84)			Altitude (m)	Vegetation / land-use	Local relief	Regional relief	Drainage
				Tmz	Latitude	Longitude					
P1	Latossolo Amarelo Distrófico petroplíntico, loamy/clayey texture, moderate A horizon	Pisoplinthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,014,036	724,711	161	natural forest and woodland	flat	plain and medium-gradient valley	well drained
P2	Latossolo Amarelo Distrófico petroplíntico plintossólico, loamy/gravelly clayey texture, moderate A horizon	Pisoplinthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,014,248	724,461	131	extensive grazing	flat	plain	well drained
P3	Latossolo Amarelo Distrófico plintossólico, clayey texture, moderate A horizon	Plinthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,023,028	727,446	138	extensive grazing	flat	plain and medium-gradient valley	weakly drained
P4	Latossolo Amarelo Distrófico argissólico, loamy texture, moderate A horizon	Xanthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,007,826	693,748	140	natural forest and woodland	flat	plain and medium-gradient valley	well drained
P5	Latossolo Amarelo Alumínico típico, clayey/very fine clayey texture, moderate A horizon	Xanthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,008,465	689,915	124	extensive grazing	flat	plain	well drained

To be continued…

Table 6 – Continuation

Profile	Brazilian System of Soil Classification (SiBCS)	Soil classification (WRB/ FAO)	County/ State	UTM coordinates (datum WGS84)			Altitude (m)	Vegetation / land-use	Local relief	Regional relief	Drainage
				Tmz	Latitude	Longitude					
P6	Latossolo Amarelo Distrófico argissólico, loamy texture, moderate A horizon	Xanthic Ferralsol	Boca do Acre-Lábrea/ AM	19L	9,008,197	693,849	132	extensive grazing	flat	plain	well drained
P7	Argissolo Amarelo Alumínico plintossólico, loamy/clayey texture, moderate A horizon	Plinthic Acrisol	Bujari/ AC	19L	8,913,325	589,878	206	natural forest and woodland	flat	plain and medium-gradient valley	weakly drained
P8	Luvissolo Háplico Pálico gleissólico, loamy/clayey texture, moderate A horizon	Stagnic Luvisol	Bujari/ AC	19L	8,909,854	594,185	185	extensive grazing	gently sloping	medium-gradient valley	weakly or moderately drained
P9	Plintossolo Argilúvico Alumínico gleissólico, loamy/clayey/very fine clayey texture, moderate A horizon	Stagnic Plinthosol	Bujari/ AC	19L	8,909,790	593,516	184	extensive grazing	gently sloping	medium-gradient valley	weakly drained
P10	Argissolo Amarelo Distrófico latossólico, sandy/loamy texture, moderate A horizon	Xanthic Acrisol	Manicoré/ AM	20L	9,118,204	680,379	103	natural forest and woodland	flat	plain	weakly or moderately drained

Table 6 – Continuation

Profile	Brazilian System of Soil Classification (SiBCS)	Soil classification (WRB/ FAO)	County/ State	UTM coordinates (datum WGS84)			Altitude (m)	Vegetation / land-use	Local relief	Regional relief	Drainage
				Tmz	Latitude	Longitude					
P11	Latossolo Amarelo Distrófico argissólico, loamy texture, moderate A horizon	Xanthic Ferralsol	Manicoré / AM	20L	9,118,058	679,931	81	extensive grazing	flat	plain and medium-gradient valley	moderately drained
P12	Latossolo Amarelo Distrófico argissólico, sandy/loamy texture, moderate A horizon	Haplic Ferralsol	Manicoré / AM	20L	9,153,711	672,058	103	natural forest and woodland	flat	plain and medium-gradient valley	well drained
P13	Latossolo Amarelo Distrófico argissólico, loamy/clayey texture, moderate A horizon	Xanthic Ferralsol	Manicoré / AM	20L	9,152,926	671,865	84	extensive grazing	flat	plain and medium-gradient valley	well drained

Table 7. Kruskal-Wallis chi-squared test (Bonferroni corrected p-values) comparing selected soil variables between land uses (forest x pasture), forests (between forests by sites) and pastures (between pastures by sites).

Source of variation	Comparison	Silt	Base saturation (BS%)	Al saturation	pH	Ca+Mg	Sum of bases
		$\chi2$ = 51.62	$\chi2$ = 46.86	$\chi2$ = 45.34	$\chi2$ = 43.37	$\chi2$ = 49.96	$\chi2$ = 49.59
Land-use within	BUJ	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	BAC	n.s.	0.001	0.001	0.001	0.001	0.001
	MAN	n.s.	0.001	0.001	0.001	0.002	0.001
Forest between	BUJ x BAC	0.007	0.002	0.003	0.002	0.003	0.002
	BAC x MAN	0.001	0.006	n.s.	0.001	n.s.	0.001
	BUJ x MAN	0.002	0.002	0.001	0.001	0.002	0.001
Pasture between	BUJ x BAC	0.001	0.001	0.001	0.001	0.001	0.001
	BAC x MAN	0.001	n.s.	n.s.	n.s.	n.s.	n.s.
	BUJ x MAN	0.001	0.007	n.s.	0.001	n.s.	0.001

Table 8. PERMANOVA testing the effects of land-use and sites on the bulk soil prokaryotic metacommunity; Land-use (forest x pasture), forest (between forests by sites) and pasture (between pastures by sites).

Source of variation	Comparisons	F	p-value
Land-use within	BUJ	4.86	0.001
	BAC	9.67	0.001
	MAN	3.60	0.002
Forest between	BUJ x BAC	9.93	0.001
	BAC x MAN	8.11	0.001
	BUJ x MAN	3.72	0.001
Pasture between	BUJ x BAC	7.81	0.001
	BAC x MAN	7.52	0.001
	BUJ x MAN	6.06	0.001
(interaction)	land-use x sites	3.97	0.001

Table 9. Generalized additive model outputs testing the correlation of each of the selected soil variables on the soil prokaryotic metacommunity structuring.

		Deviance explained (%)	F	p-value
Soil chemical variables	pH	91.6	61.92	0.001
	base saturation (BS%)	93.9	88.28	0.001
	Al saturation	95.2	112.7	0.001
	Ca + Mg	80.2	22.62	0.001
Soil physical variable	silt	76.9	18751	0.001

Table 10. PERMANOVA testing the effect of sites on the litter and rhizosphere prokaryotic metacommunities.

Source of variation	Comparisons	F	p-value
Litter between	BUJ x BAC	2.69	0.002
	BAC x MAN	4.82	0.001
	BUJ x MAN	4.10	0.001
Root layer between	BUJ x BAC	15.94	0.001
	BAC x MAN	15.04	0.001
	BUJ x MAN	9.75	0.001

Table 11. Kruskal-Wallis chi-squared test for scales of diversity between the forest floor (litter, root layer, and bulk soil) and pasture bulk soil.

	α - diversity		β - diversity		γ - diversity	
	χ2	p-value	χ2	p-value	χ2	p-value
BUJ	0.109	0.743	16.916	< 0.001	6.648	0.009
BAC	6.607	0.014	9.967	< 0.01	0.411	0.521
MAN	2.632	0.104	12.405	< 0.001	0.172	0.678

8.3 Chapter III Supplements

Table 12. Characterization of soil physical and chemical variables of HSL and LSL pastures across Amazonian forest-to-pasture conversion landscapes; Mean values and standard deviation of 20 samples (per severity level).

Variable	Unit	High Severity Level (HSL)		Low Severity Level (LSL)	
CDW	g kg^{-1}	230.90	± 52.21	208.80	± 106.59
clay	g kg^{-1}	251.00	± 77.08	272.40	± 129.08
degree of flocculation	%	14.85	± 9.35	22.95	± 13.97
silt:clay ratio	-	0.46	± 0.25	2.00	± 1.22
silt	g $k^{-1}g^{-1}$	136.40	± 66.08	439.80	± 178.14
coarse sand	g kg^{-1}	283.25	± 225.10	38.40	± 17.80
Al	cmolc kg^{-1}	0.70	± 0.63	1.76	± 2.28
C	g kg^{-1}	15.87	± 2.98	16.81	± 3.67
Ca+Mg	cmolc kg	1.91	± 1.35	4.46	± 4.10
C:N ratio	-	10.20	± 1.11	8.15	± 1.14
H	cmolc kg^{-1}	5.50	± 1.75	6.59	± 2.12
H + Al	cmolc kg^{-1}	6.19	± 2.27	8.34	± 4.24
K	cmolc kg^{-1}	0.14	± 0.10	0.19	± 0.14
Al saturation (m%)	%	30.05	± 27.04	33.85	± 33.59
N	g kg^{-1}	1.57	± 0.30	2.06	± 0.49
Na	cmolc kg^{-1}	0.02	± 0.01	0.08	± 0.15
P	mg kg^{-1}	3.00	± 3.32	4.35	± 2.83
pHH_2O	-	5.10	± 0.71	5.18	± 0.55

To be continued…

Table 12 – Continuation

Variable	Unit	High Severity Level (HSL)		Low Severity Level (LSL)	
sum of bases (SB)	cmolc kg^{-1}	2.07	± 1.41	4.72	± 4.18
CEC effective	cmolc kg^{-1}	8.26	± 1.51	13.07	± 4.07
base saturation (BS%)	%	26.20	± 18.18	34.35	± 24.97
Cu	mg kg^{-1}	0.57	± 0.43	1.20	± 0.31
Fe	mg kg^{-1}	125.20	± 75.16	162.70	± 112.45
Mn	mg kg^{-1}	13.62	± 14.95	116.81	± 109.65
Zn	mg kg^{-1}	0.80	± 0.59	2.68	± 2.03

Table 13. Characterization of bromatological variables in the forage of HSL and LSL pastures across Amazonian forest-to-pasture conversion landscapes; Mean values and standard deviation of 20 samples (per severity level).

Variable	Unit	High Severity Level (HSL)		Low Severity Level (LSL)	
Ether extract	%	1.75	± 0.55	1.40	± 0.36
Nitrogen-free extract	%	48.59	± 3.25	45.90	± 4.56
Acid detergent fiber	%	38.33	± 2.51	37.36	± 1.35
Neutral detergent fiber	%	66.26	± 4.29	64.27	± 2.92
Mineral matter	%	7.68	± 1.64	9.03	± 0.89
Total digestible nutrients	%	53.50	± 2.12	54.25	± 0.85
Total nitrogen (TN)	%	1.55	± 0.36	1.46	± 0.46
Non-protein nitrogen	% TN	26.19	± 15.36	21.62	± 1.63
Crude protein (CP)	%	9.65	± 2.24	9.14	± 2.90
Indigestible acid detergent fiber	%	1.13	± 0.58	1.16	± 0.22
Indigestible acid detergent fiber	% CP	12.40	± 6.20	13.54	± 3.77
Indigestible neutral detergent fiber	%	3.45	± 1.05	3.45	± 1.04
Indigestible neutral detergent fiber	% CP	38.75	± 15.93	38.03	± 7.06
Non-protein nitrogen (ProtA)	% total N	26.20	± 15.36	21.62	± 1.63
Soluble protein (ProtB1)	% total N	8.24	± 1.69	10.53	± 8.00
Insoluble protein (ProtB2, intermediate)	% total N	26.82	± 6.13	29.83	± 6.61
Insoluble protein (ProtB3, slow)	% total N	26.35	± 12.09	24.49	± 6.06
Indigestible protein (ProtC)	% total N	12.40	± 6.20	13.54	± 3.77
Rumen-degradable protein	% CP	31.25	± 9.13	31.50	± 14.66
Soluble protein	% CP	34.44	± 13.83	32.15	± 8.47
Ca	g kg^{-1}	6.44	± 2.34	6.99	± 1.42
K	g kg^{-1}	15.05	± 2.68	14.65	± 4.45
Mg	g kg^{-1}	2.36	± 0.49	3.33	± 0.79
N	g kg^{-1}	15.44	± 3.58	14.63	± 4.64
Na	%	0.08	± 0.01	0.07	± 0.00
P	g kg^{-1}	0.89	± 0.11	1.27	± 0.25
Cu	mg kg^{-1}	4.50	± 0.89	5.00	± 0.73

To be continued…

Table 13 – Continuation

Variable	Unit	High Severity Level (HSL)		Low Severity Level (LSL)	
Fe	mg kg^{-1}	206.50	± 141.59	229.25	± 74.68
Mn	mg kg^{-1}	138.75	± 64.23	391.00	± 278.54
Zn	mg kg^{-1}	12.50	± 1.54	20.00	± 7.64

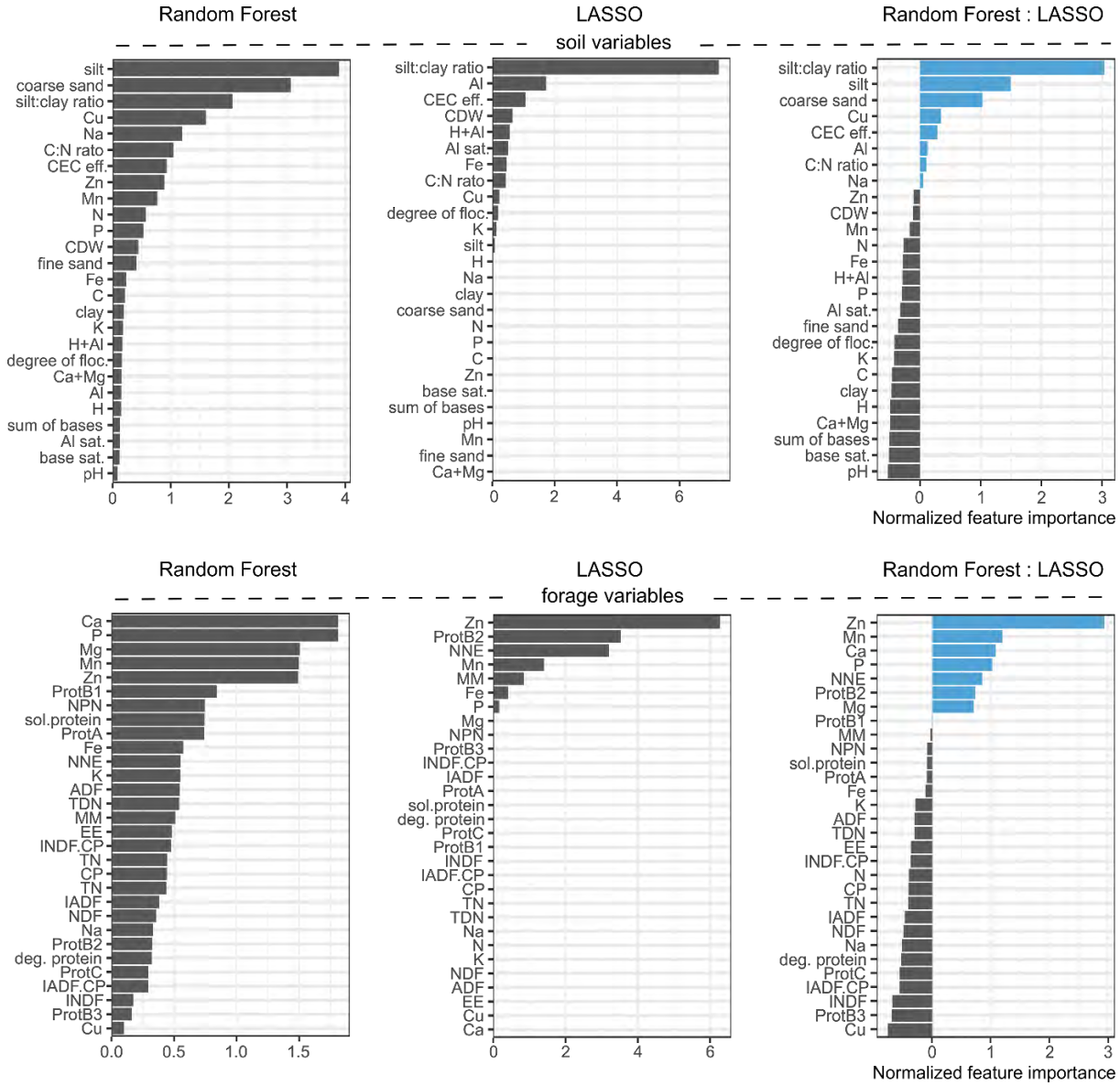

Figure 43. Variable selection of abiotic factor in soil (upper box) and forage (bottom box) from pastures of HSL and LSL systems. Individually is shown the selected variables by Random Forest and LASSO and the variables with the highest normalized feature importance retained by the integrated Random Forest : LASSO model.

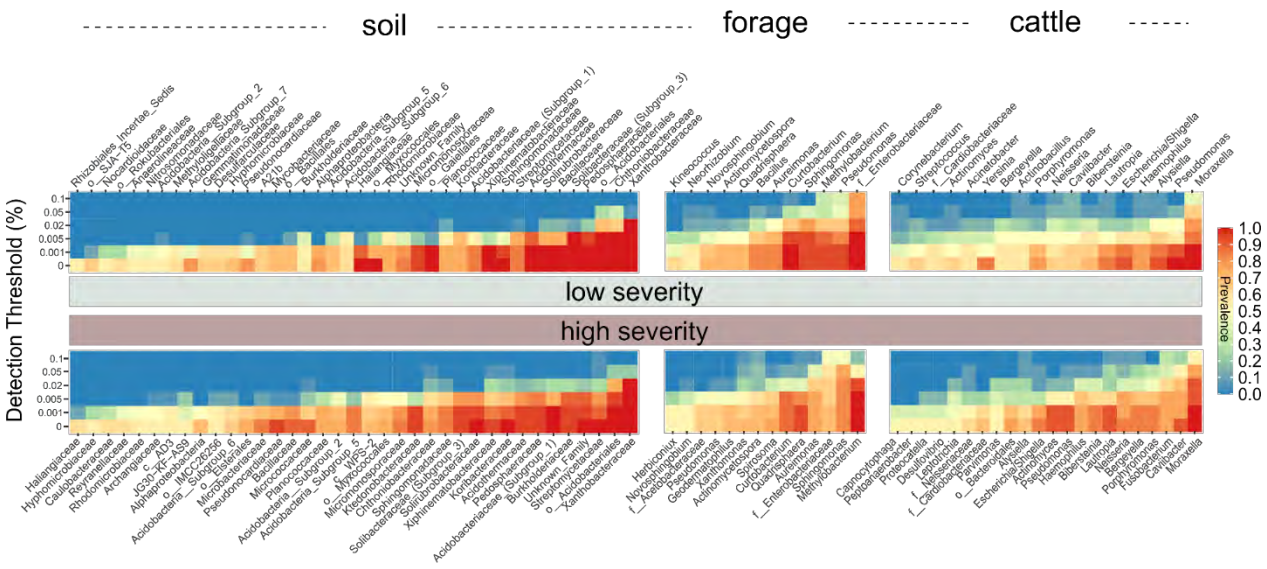

Figure 44. Heatmap with taxa ordered decreasingly according to their highest persistence and abundance in DNA samples from soil, forage, and cattle from HSL and LSL systems.

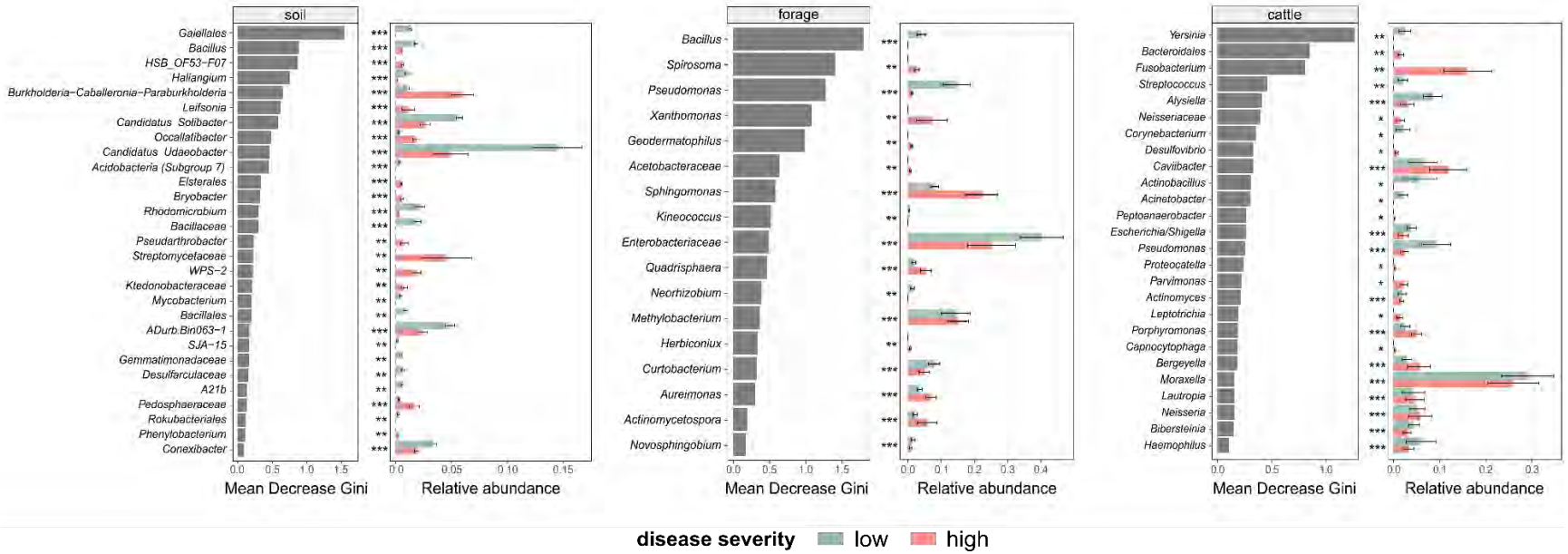

Figure 45. Differential abundance analysis by Random Forest. The analysis ranks taxa (genus level) according to the average decrease in Gini weights and shows relative abundance in the HSL and LSL systems. (***) significant at $p < 0.001$, (**) $p < 0.01$, (*) $p < 0.05$, n.s. not significant. Error bars indicate the ± standard error (SE).

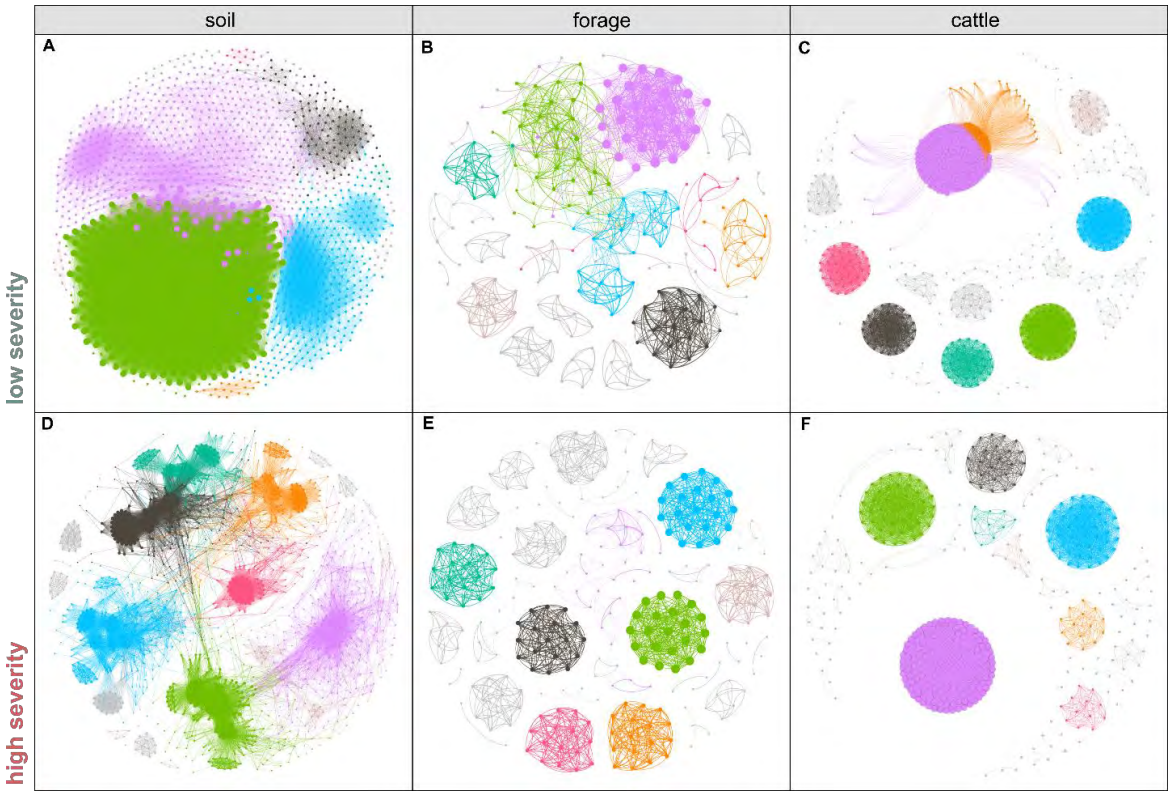

Figure 46. Co-occurrence networks from the abundance matrix of soil, forage, and cattle prokaryotes from the HSL and LSL systems. Networks were constructed from taxa that showed at least one strong (Spearman ≥ 0.70) and statistically significant ($p < 0.001$) correlation.

Table 14. Topologies of co-occurrence networks based on abundance matrix of core microbiota from soil, forage, and cattle of HSL and LSL systems.

Topological measures	Core microbiota sub-communities					
	Soil		Forage		Cattle	
	LSL	HSL	LSL	HSL	LSL	HSL
Nodes [a]	66	58	19	25	22	34
Edges [b]	631	183	136	244	200	446
Average weighted degree [c]	14,226	4,741	5,172	7,247	6,826	9,324
Network diameter [d]	4	9	2	2	2	2
Graph density [e]	0.294	0.111	0.795	0.813	0.866	0.795
Modularity [f]	0.217	0.431	0.108	0.149	0.155	0.170
Number of communities [g]	4	5	2	2	3	3
Average clustering coefficient [h]	0.687	0.566	0.794	0.820	0.866	0.792

a Number of features with high significant biserial correlation coefficients ($p < 0.05$); b Number of connections obtained by pairwise correlations between nodes; c The average number of connections per node in the network, corrected according to the proportion of nodes and edges in the network; d The longest distance between nodes in the network, measured in number of edges; e Measures how much each node in a graph tend to cluster together; f The capability of the nodes to form a modular structure, that is, a structure with high density of nodes and clustered topology. g A community is defined as a group of nodes densely connected internally.

9.4 Chapter IV Supplements

9.4.1 Access link with input data for use in Hydrus 1D

ROCHA, F. I. Meteorological Data_Hydrus1D input.csv. (2021) - Google Drive link

9.4.2 Workflow for generating the simulations for this study using HYDRUS-1D software:

a) Main processes

- Simulate “Water Flow” and “Root Water Uptake”.

b) Geometry information

- Number of Soil Materials: 2 (indicates a two-layered soil profile)
- Number of Layers for Mass Balance: 2 (mass balances will be calculated for each soil layer)
- Decline from Vertical Axes: 1
- Depth of the Soil Profile (cm): 100

9.4.3 Time information

a) Time discretization

- Initial Time (day): 0
- Final Time (day): 2922
- Initial Time Step (day): 0.001
- Minimum Time Step (day): 1e-005
- Maximum Time Step (day): 5
- Check Time-Variable Boundary Conditions
- Number of Time-Variable Boundary Records: 2922

9.4.4 Print information

- Number of Print Times: 3

9.4.5 Water flow

a) Iteration criteria

- Default values

b) Soil hydraulic model

- van Genuchten – Mualem

c) Hysteresis

- No hysteresis

d) Soil hydraulic parameters

- Should be used the soil hydraulic parameters found on **Table 3.**

e) Boundary conditions

- Upper Boundary Condition: Atmospheric BC with Surface Layer
- Lower Boundary Condition: Free drainage; h=0
- Initial Condition: In Pressure Heads
- Max h at Soil Surface: 1 cm

9.4.6 Root

a) Water uptake model

- Water Uptake Reduction Model: Feddes
- Solute Stress Model: No Solute Stress
- Critical Stress Index for Water Uptake: 1

b) Water uptake parameters

- Database: Pasture (WESSELING, 1991)

9.4.7 Time variable boundary conditions

- Fill out the table in the Time-Variable Boundary Conditions dialog window with the provided in: ROCHA, F. I. Meteorological Data_Hydrus1D input.csv. (2021)

9.4.8 Profile information

Profile discretization: Node of observation 0, 10, 30, and 100 cm
Material distribution: Minimum at 10 cm, and Maximum at 11 to 100 cm
Root linear distribution:

00 – 10 cm (40%)
11 – 20 cm (20%)
21 – 40 cm (10%)
41 – 60 cm (5 to 0; unselect the function “Use top value for both”)