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Effect of the conversion of conventional pasture to intensive silvopastoral systems on edaphic bacterial and ammonia oxidizer communities in Colombia

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ABSTRACT

Colombia, as well as many tropical countries, has experienced severe deforestation in the last decades, and millions of acres of native forest areas (F) have been replaced by conventional monoculture pastures (CP), contributing to ecological and soil degradation. In response, multi-canopy intensive silvopastoral systems (ISS), which includes herbs, shrubs and trees, have been developed to provide local fodder sources for livestock while reducing the need for external inputs with a goal to conserve landscapes and improve soil quality. However, there is limited information on the temporal responsiveness of ISS to deliver ecosystem services as reflected in soil microbial properties. Therefore, the objective of this study was to examine the shifts of total and ammonia-oxidizing bacteria (AOB) communities along an ISS chronosequence (ranging from 3 to 15 years since establishment), in comparison to CP and native F and investigate *P. juliflora* trees as a resource island relative to soil microbial properties. Denaturing gradient gel electrophoresis (DGGE) fingerprints of 16S rRNA gene (total bacteria) as well as *amoA* gene (ammonia-oxidizing bacteria) (AOB) indicated that soil bacterial communities varied between the land uses, with higher similarities between F and ISS communities, in comparison to CP. The abundance and nitrification potential of ammonia oxidizers were significantly higher in CP and lower in F. In addition, the bacterial communities across ISS chronosequence were more similar between older (ISS-12) and intermediate (ISS-8) systems in comparison with youngest systems (ISS-3). Finally, the canopy of *P. juliflora* tree did not have an impact on structure of total bacterial community; though, it did have an effect on the structure of AOB communities. Our study suggests that ISS might restore some of the ecosystem services offered by soil microbial communities.

1. Introduction

Deforestation in tropical countries of the Americas have affected millions of acres of natural forest (F). These unique ecosystems have been mainly transformed to livestock or agriculture activities, to ensure food security and respond to demographic growth [1–3]. In Colombia, the livestock sector accounts for 4% of the national economy [4] and occupying 40 million hectares, which is 35% of total area of the country [5]. Furthermore, these systems causes deforestation of 300,000 ha per year [3]. Conventional pastures (CP) often include practices such as monocultures that significantly

Abbreviations: ISS, Intensive Silvopastoral System; ISS-12, Older Intensive Silvopastoral System (12–15 years old); ISS-8, Intermediate Intensive Silvopastoral System (8–10 years old); ISS-3, Young Intensive Silvopastoral System (3–6 years old); F, Native forest; CP, Conventional monoculture pastures.

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reduce biodiversity, soil tillage, and application of agrochemicals, which contribute to the deterioration of the physical, chemical and biological properties of soil [3].

Intensive silvopastoral systems (ISS) are a specialized form of agroforestry, which were developed in Colombia and Mexico at a few pilot farms in response to environmental problems and low sustainability of conventional livestock activities [6,7]. In contrast to CP, ISS livestock production systems integrate trees, high densities of shrubs and grasses, while tillage and agrochemical inputs are no longer required [6,7]. The introduction of shrubs and trees to pastoral systems increases the above and below ground biomass that provides greater input of organic material and other nutrients to the soil. Additionally, profound roots from trees can move water and nutrients from deeper soil layers to the surface generating resource islands [8,9]. Similarly, the lack of aeration by tillage reduces degradation rate of soil organic matter, and improves soil structure. A higher content of organic matter, improves other soil properties such as cation and anion-exchange properties, water holding capacity, porosity, aeration and infiltration. Such soil is more fertile and has higher resistance to compaction and erosion, mitigating the effect of livestock trampling [10,11].

The inclusion of shrubs and trees offers richer alimentation to the livestock and provides shade that reduces animal stress as well as soil water evaporation. Additionally, greater vegetative diversity contributes to wild life diversity of birds, insects and other organisms who can act as natural pest controlling agents. Furthermore, ISS typically have leguminous species such as *Leucaena leucocephala,* which can perform symbiotic nitrogen fixation that would provide a limiting nutrient for the fodder plants that feed livestock and therefore provide a means to reduce the need to purchase N fertilizer for these systems. In summary, ISS would be expected to improve soil properties, reduce the need of external fertilizers, pesticides and irrigation, contribute to carbon sequestering, minimize livestock's heat stress and diversify its diet, and help to preserve biodiversity, all this combined with economic benefits to the farmer [12,13].

Hatico Natural Reserve in Valle del Cauca department of Colombia is one of the principal pilot farms in which ISS have been developed [6,7]. Since 1993, the farm owners have gradually converted their CP into intensive silvopastoral systems and conducted numerous studies about its productivity, birds and insect's biodiversity, carbon emission and sequestration, and soil physicochemical and biological properties [14,15]. In addition, these systems had different start dates for converting from CP to ISS that has enabled time course studies on ISS using the established chronosequences. For this reason, Hatico Natural Reserve is a good model to study the effect of the establishment of intensive silvopastoral systems on soil bacterial communities.

To understand the effect of land uses on soil quality, it is important to consider physical, chemical and biological parameters [16,17]. It is widely accepted that microbial communities have a crucial role in nutrient cycling, soil health, carbon sequestration, plant growth promotion and pest control [18]. Nevertheless, it is currently not clear which is the relationship between a specific microbial community and soil quality and furthermore, there is no simple research approach to address this relationship [19]. This is because there is no comprehensive mechanistic understanding of the complex relationship between soil microbial community and soil function [20]. One strategy to circumvent this problem is to compare soil microbial communities at a disturbed or degraded site with that of an adjacent site that is natural or managed to maintain or increase soil quality [19].

The microbiological indicators of soil quality include microbial biomass, enzymatic activity, the structure of the microbial communities or specific functional groups such as methanogens, methanotrophs, nitrogen (N) fixing bacteria, denitrifying and nitrifying bacteria [17,19,21,22]. The use of specific functional and sensitive groups, such as ammonia-oxidizing bacteria is a good option since these bacteria are directly related to a specific soil function [23,24] and they might respond faster to environmental changes increasing the sensitivity of soil quality assessment [25].

Nitrogen cycling in soil, involving diverse bacterial groups is one of the most important ecosystem processes, since N is a major limiting nutrient for plant growth, and the ratio between ammonia and nitrate can affect plant community structure [26]. The oxidation of ammonia to nitrite performed by ammonia-oxidizing bacteria (AOB) and archaea (AOA) is the initial and rate-limiting step in nitrification. It reduces the ratio between ammonium and nitrate but also can lead to denitrification and N loss from soil [27,28]. AOB generally represents a small fraction of the total bacterial communities in soil, and due to their low physiological tolerance and high sensitivity to environmental disturbances, they are sensitive indicators for disturbances caused by land managements (e.g., tillage, herbicide and fertilizer additions) [29,30].

Previous studies of soil microbial communities in ISS at the Hatico Natural Reserve showed that the ISS system increased soil microbial biomass, enzymatic activity, and microbial diversity (FAME profiling), decreased microbial stress and compaction over CP [13,31]. Furthermore, FAME profiles suggested that microbial community of ISS soils had higher similarity to those of F than to the microbial communities of CP soils [31]. The latter study also showed that the inclusion of *P. juliflora* (Sw.) DC trees, contributes to the formation of fertile islands with higher nutrient and microbial biomass concentration and enhanced enzymatic activities. Thus, the results of these studies indicate that ISS improves soil quality and metabolic function.

The current study builds on these findings by applying DNA fingerprinting (PCR-DGGE) using 16S rRNA gene (total bacterial communities), and to expand our knowledge about the abundance, potential activity of ammonia oxidizers (AO), and community structure of AOB. Then, the objectives were to investigate the effect of livestock land use systems (F, CP and ISS chronosequence: 3–15 years) as well as the presence of a dominant tree in the ISS (*P. juliflora*) on total bacterial and AOB community structure, AO density and the nitrification potential.

2. Materials and methods

2.1. Site description and experimental design

The study was conducted at El Hatico Natural Reserve, located in the municipality of El Cerrito in the flatlands of the Cauca river (Valle del Cauca- Colombia, 3° 47′ N, 76° 16′ W). The experimental design was a completely randomized design with five treatments: 1) forest (F); 2) Intensive Silvopastoral Systems (ISS) of 12–15 years old (ISS-12) 3) ISS of 8–10 years old (ISS-8); 4) ISS of 3–6 years old (ISS-3) and 5) conventional pastures (CP). Each treatment had three spatially separated replicates and, except for F, each treatment was subdivided into grazing paddocks. For ISS chronosequence and CP, each replicate had between 0.6 and 0.8 ha^{-1} divided into 12–14 grazing paddocks. The tropical dry forest is situated within "El Hatico" and is over 80 years old with minimal human intervention. The 30 years old CP systems are a monoculture of native grass (*Cynodon plectostachyus*), have no tree cover and are intensively managed using tillage, fertilizer, pesticides and irrigation. The ISS, which is managed without tillage, fertilizer or pesticide application, contains three-layered strata. The ground level consist of herbaceous forage (all system have *Cynodon plectostachyus* while ISS-3 and ISS-8 have also *Paniccum maximum* var. Tanzania and Mombasa grasses); the intermediate level consist of high density of