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Soil microbial communities under cacao agroforestry and cover crop systems in Peru

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ABSTRACT

Cacao (*Theobroma cacao*) trees are grown in tropical regions worldwide for chocolate production. We studied the effects of agroforestry management systems and cover cropping on soil microbial communities under cacao in two different replicated field experiments in Peru. In the first experiment, two agroforestry systems, Improved Traditional Agroforestry System (ITAS) and Improved Natural Agroforestry System (INAS), were compared. ITAS was a 'slash and burn' system in which all native vegetation was removed prior to replanting with cacao and other trees while INAS used selective removal of uneconomical trees followed by cacao planting. Soil microbial communities were analyzed by phospholipid fatty acid (PLFA) analysis and terminal restriction fragment length polymorphism (TRFLP) analysis. Soils in the ITAS system had altered microbial community structure and a lower Gram-negative to Gram-positive ratio when compared to soils in the INAS system. However, soil microbial community structure was also affected by a large soil pH gradient (three pH units) across this experiment. In the cover crop experiment, five cover crops, *Arachis pintoi* (perennial peanut), *Calopogonium mucunoides* (calopo), *Canavalia ensiformis* (jackbean), *Centrosema macrocarpum* (centro), and *Callisia repens* (callisia), and two controls (one with and one without nitrogen fertilization), were compared. Cover cropping with centro or perennial peanut increased the Gram-negative to Gram-positive ratio, while centro reduced the fungal biomass. Microbial community structure was significantly affected by cover cropping. Our results indicate that management systems and cover cropping can affect soil microbial community structure in tropical agroforestry systems, but the effects of soil edaphic properties must be considered as well.

1. Introduction

Cacao trees (*Theobroma cacao*) are grown for cocoa production on an area of over 10 million hectares (ha) in tropical regions throughout the world (FAOSTAT, 2016). Eighty-three percent of cocoa is currently produced in Africa with Asia/Oceania and the Americas producing the remainder (FAOSTAT, 2016). There are approximately 5–6 million cocoa farmers worldwide, growing cacao on typically small (less than 5 ha) family-run farms (World Cocoa Foundation, 2014).

Cacao is an under-story shade-tolerant tree, but it can be grown under a range of conditions from heavy shade to full sun. In agroforestry systems, partial shade is provided by thinning the natural forest vegetation or by clearing the forest and then planting one or more species of shade trees. Full sun is provided by clearing the forest and then planting only cacao. Each of these options results in different levels of plant and animal diversity (Hartemink, 2003; Sonwa et al., 2007), and cacao monoculture under full sun has been shown to be less sustainable and more prone to fungal diseases than shaded cacao production (Rice and Greenberg, 2000).

Nutrient requirements of cacao in agroforestry systems are quite different than those grown in open canopy. In agroforestry systems light levels and soil conditions affect nutrient availability and cacao tree nutrition. Enhanced cacao production in agroforestry systems results from efficient nutrient cycling, improved soil characteristics, modified light infiltration, enhanced moisture availability and reduced weed competition (Ahenkorah et al., 1987; Beer et al., 1997; Hartemink, 2005). Agroforestry systems differ from monoculture systems in partitioning of resources, synchrony of resource use, and the ability of each species to capture and cycle nutrients (Ewel and Bigelow, 1996; Isaac et al., 2007; Schroth et al., 2001). Cacao farms often receive minimal or no fertilization, and nutrients are exported from the system through harvest, leaching, and runoff (Holscher et al., 1996; Sommer et al.,

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2004). Agroforestry systems can play an important role in improving soil fertility by sequestering organic carbon in the soil, augmenting N input through nitrogen fixation, reducing soil loss by erosion, cycling nutrients through litter fall, and retrieving nutrients from deeper soil horizons (Hartemink, 2003).

Cover crops have been incorporated into cacao management systems. Beneficial effects of cover crops have been reported in temperate and tropical agricultural management systems, including increased soil organic matter content, higher levels of available nutrients, increased concentration of nutrients at surface layers, reduced leaching losses of nutrients, better erosion control, improved soil structure and texture, decreased soil acidity and compaction, reduced fertilizer input costs, improved water holding capacity, increased biological activities, weed suppression, decreased disease, and reduced pest problems (Baligar and Fageria, 2007; Fageria et al., 2010; Teasdale, 1998).

Terrestrial ecosystems, including agricultural and agroforestry systems, are dependent on ecosystem services provided by soil microbes, including soil formation, soil aggregation, nutrient cycling, biocontrol of pathogens, and degradation of xenobiotics (Lehman et al., 2015b). Soil microbial activity is an important factor in soil quality and health (Doran and Zeiss, 2000; Lehman et al., 2015a). Soil microbial communities are closely linked to plant communities, with plants providing most microbes energy through root exudates and plant litter (Schenck zu Schweinsberg-Mickan et al., 2012; Singh et al., 2016), and plant speciation is one of several factors that control soil microbial communities (Cline and Zak, 2015; Mitchell et al., 2012).

In this study we compared soil microbial biomass and community structure, including Archaea, Bacteria, and fungi, under two cacao agroforestry systems in Peru. In a separate experiment, we also compared the effects of five different cover crops on soil microbial biomass and community structure in one agroforestry system. We tested the following hypotheses: (1) Agroforestry management systems affect soil fertility, microbial biomass and community structure; and (2) Cover cropping increases soil fertility and soil microbial biomass while altering soil microbial community structure.

2. Materials and methods

2.1. Site description

The study area was located in the Department of San Martin, Province of Tarapoto, District of La Banda de Shilcayo in the El Choclino sector of Peru. The experiments were located at the Choclino farm of the Instituto de Cultivos Tropicales (ICT) (6° 28′ 37.3″ S; 76° 19′ 54.6″ W), at an altitude between 500 and 530 m above sea level. In accordance with the Peruvian ecological map, the study site is located within the living area of Pre-Mountain Tropical Dry Forest (Holdridge, 1971). The terrain is steep in some areas with slopes greater than 50%, but mostly dominated by a slope of less than 50%. The primary vegetation was a secondary forest native to the region approximately 25 years old, with forest species (58%), palm (3%), and lianas and herbaceous (39%) plants (Arévalo-Gardini et al., 2015). In the experimental area, the average annual rainfall is 1250 mm with an average temperature of 26 °C and an average relative humidity of 87%.

2.2. Experimental design

2.2.1. Agroforestry experiment

The soil under the experiment is Order Alfisol, Suborder-Ustafl (Soil Survey Staff, 2010). The texture of the surface soil (0–20 cm) varies from clay to sandy loam, with mean values of 27% sand, 26% silt, and 47% clay. Soil chemical properties are presented in Table 1.

Details of this experiment have been previously described (Arévalo-Gardini et al., 2015). Briefly, in 2004 land was prepared for installation of two long term cacao management experiments: Improved Native Agroforestry Systems (INAS) and Improved Traditional Agroforestry System (ITAS). The primary difference between these two systems is that in the INAS system, uneconomical trees were selectively removed to reduce tree density to achieve approximately 50% shade, while in the ITAS system all vegetation was removed by the traditional slash and burn method.

Table 1
Soil chemical properties of agroforestry system experiment (n = 36). Values are reported as means of all cacao genotypes as genotype had no significant effects or interactions. All values are least square means ± standard deviation, and values within a row with different letters are significantly different ($P < 0.05$, Bonferroni's test).

Property	System	
	INAS	ITAS
pH	5.33 ± 1.09 A	4.92 ± 1.09 A
EC[a]	286.7 ± 206.1 A	161.7 ± 206.1 A
Total C[b]	2.81 ± 1.10 A	2.34 ± 1.10 A
Total N[b]	0.23 ± 0.08 A	0.19 ± 0.08 A
NH_4^+ N[c]	5.03 ± 1.35 A	6.65 ± 1.35 A
NO_3^- N[c]	5.94 ± 3.10 A	2.91 ± 3.10 B
P[d]	5.78 ± 3.41 B	10.60 ± 3.41 A

[a] μS/cm.
[b] %.
[c] mg/kg.
[d] mg/kg (Mehlich 3 extractable P).

In both systems, cacao rootstock IMC-67 was planted in 2005 at a density of 1768 plants/ha. Shade trees were also planted using native species of the area such as *Inga* spp. (shimbillo), *Macrolobium acaciafolium* (pashaco), *Calycophyllum spruceanum* (capirona), *Cedrelinga cateaniformes* (tornillo), and *Vitex pseudolia* (paliperro). Field plots were laid out in a split plot design with 3 replicate plots per system. Each plot was 100 × 54 m and divided into 60 subplots to accommodate 60 different cacao genotypes. Each subplot measured 10 × 6 m to accommodate 10 plants of each genotype. Well-developed disease free shoot cuttings from 59 cacao genotypes were side grafted on 6 month old rootstock. In this paper we report on 6 of those genotypes, CCN-51, ICS-95, UF-613, ICT-2171, ICT-1112, and ICT-2142, the first three representative of genotypes commonly grown in Peru and the last three new genotypes selected on farmers' fields which have shown potential for high yields.

2.2.2. Cover crop experiment

This experiment has been previously described (Hall et al., 2010). The soil is order Inceptisol (Soil Survey Staff, 2010), sandy clay loam, with 67% sand, 10% silt, and 23% clay. The general management protocol follows the ITAS system as described above. A split-plot design consisting of 3 replicate blocks (82 × 45 m), 7 plots (cover crop treatment) per block (45 × 10 m), and 5 subplots (cacao genotype) per plot, was used. Four perennial leguminous cover crops, *Arachis pintoi* (perennial peanut), *Calopogonium mucunoides* (calopo), *Canavalia ensiformis* (jackbean), *Centrosema macrocarpum* (centro), and one non-leguminous cover crop, *Callisia repens* (callisia), were established in November and December of 2004. Two control treatments without cover crops, using N fertilizer at 0 and 50 kg/ha, were also included. Cover crop seeds were planted at a spacing of 0.5 × 0.5 m.

Banana was established as temporary shade between December 2004 and January 2005 with a spacing of 3 × 2 m. Shade tree species *Vitex pseudolia* (paliperro) and *Calycophyllum spruceanum* (capirona) were planted at a spacing of 6 × 9 m to achieve a total of 185 plants/ha. After 2 years of cover cropping 5 cacao genotypes grafted onto rootstock IMC67 were planted at a spacing of 2 × 3 m to achieve a density of 1666 plants/ha., using 10 plants of each cacao genotype per subplot. In this paper we report on 2 of those genotypes, CCN-51 and ICS-95, which are representative of genotypes commonly grown in Peru.