ORIGINAL PAPER

Soil enzymatic activities and microbial biomass in an integrated agroforestry chronosequence compared to monoculture and a native forest of Colombia

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Abstract Large tracts of land in South American native forests have been converted to monoculture for livestock production which could negatively affect soils and environmental quality. A proposed management alternative is to use agroforestry systems, but little information is available on how they affect the soils. The objective was to assess the effect of a silvopastoral chronosequence in a tropical region of Colombia on soil microbiological and physico/chemical properties. The systems (three replications) were: monoculture grass conventional pasture (CP), native forest (F), and a silvopastoral system (SS) chronosequence with ages of 3 to 6 (SS_3), 8 to 10 (SS_8), or 12 to 15 (SS_{12}) years. Soil responses to these land management were determined by measuring soil chemical (total C and pH), physical (penetrometer resistance and bulk density), and microbiological properties (activities of ß-glucosidase, urease, and alkaline and acid phosphatase and microbial biomass). Because of differences in soil texture across management treatments, microbiological properties were normalized on organic C content basis. SS_{12} showed the highest microbial biomass and enzyme activities on a per unit C basis and was consistently and significantly different from CP. Additionally, microbiological to C ratios were significantly affected by SS establishment age ($P<0.05$). The low microbiological responses were consistent with high penetration resistance and bulk density of CP which indicates that the SS are improving soil quality. This study presented quantitative data that SS stimulated soil microbial biomass and enzyme activities, which indicates greater potential to carry out biogeochemical process, and that SS provides a more favorable microbial habitat.

Keywords Soil management · Enzyme activities · Agroforestry systems · Silvopastoral systems · FAME

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Introduction

Currently, there is widespread interest in developing sustainable agricultural systems that are less dependent on external inputs, especially fertilizers and herbicides, to reduce impacts on the environment and conserve and improve soils (Moonen and Bàrberi 2008). In Latin America, conventional monoculture pastures (CP) have replaced the tropical forests, significantly reducing biodiversity and carbon (C) sequestration in plant biomass and soils (Murgueitio 2004; Chará and Murgueitio 2005; Pagiola et al. 2007), increased erosion, and bringing about other negative environmental impacts (FAO 2005).

One proposed approach to overcome the ecological limitations of CP are agroforestry systems that increase plant species diversity and have a multi-layered forest canopy (Garrity 2004; Udawatta et al. 2008; Nair et al. 2009; Udawatta et al. 2009). The silvopastoral system (SS) is a specific type of agroforestry designed for livestock production which combines grass/leguminous herb species with woody shrub and tree species to provide forage and ecological benefits (Montagnini et al. 2003; Isaac et al. 2005; Reis et al.

2009). Previous studies in other regions have demonstrated that SS increases dry season forage and milk production, enhances soil nutrient accumulation and soil microbial biomass C and N, and promote soil quality compared to sole cropping systems (Arevalo et al. 1998; Amatya et al. 2002; Menezes et al. 2002; Montagnini et al. 2003; Tiessen et al. 2003). However, there is relatively little information on SS in South America, particularly in the northern region.

Soil microorganisms control ecosystem functioning as mediators of decomposition, C stabilization, and nutrient cycling (Coleman and Whitman 2005). Thus, an important factor to consider before widespread adoption of putatively improved ecosystems is to evaluate and understand their impacts on soil biology and biochemistry as indicators of their ability to deliver ecosystems services (Doran and Parkin 1994; Van Bruggen and Semenov 2000).

Some enzyme activity assays have been shown to be early and sensitive indicators of soil management (Bandick and Dick 1999; Ndiaye et al. 2000). Research on sterilized soils without viable organisms has shown a significant enzyme activity (typically 40% to 60% of the enzyme activity of non-sterile soil) due to enzymes stabilized by the soil matrix (Skujins 1978; Knight and Dick 2004). Therefore, the present enzyme assay determines both the activity of the viable cells and the long-term accumulation of enzymes stabilized in the soil. Ecosystem management that promotes accumulation of enzymes in soils should favor organic matter accumulation which in turn promotes aggregation and soil structure. The combination of enzyme activities and microbial biomass measurement has been widely used over the last 10 years to study the microbiological responses to agricultural management (Wick et al. 2000; Clegg et al. 2006; Bastida et al. 2007; Acosta-Martínez et al. 2008; Jangid et al. 2008; Meriles et al. 2009; Sotomayor-Ramírez et al. 2009; Udawatta et al. 2009).

However, relatively little information is available on SS and soil quality under tropical and subtropical conditions in developing countries (Balota et al. 2004). An experimental site within a commercial livestock operation in Colombia provided a unique opportunity to investigate a replicated silvopastoral chronosequence in comparison to a traditional grass monoculture pasture system. Therefore, the objectives were to study physicochemical and microbiological responses to: (1) three land management systems (CP, native forest, or SS) and (2) stand age (3–6, 8–10, or 12–15 years) of SS.

Materials and methods

Experimental site description and design

The study was conducted at the Hatico Natural Reserve located in the municipality of El Cerrito—in the department

of Valle del Cauca, Colombia (3°47′ N, 76°16′ W). The site is 1,000 m above sea level with an average temperature of 24°C. There are two rainy seasons, March to May and October to November. Average annual rainfall is 750 mm, relative humidity is 75%, and average annual evaporation is 1825 mm/year. The soils are a mixture of volcanic ash and thick alluvial deposit and classified as mollisols in the USDA soil classification system (USDA 2006). The area started to be converted from native forest to livestock production about 80 years ago.

The study had a completely randomized design with five treatments of: (1) CP, (2) native forest (F), and the silvopastoral chronosequences of (3) 3 to 6 (SS_3), (4) 8 to 10 (SS_8), and (5) 12 to 15 (SS_{12}) years. Each treatment had three spatially separated replicates. Except for F, each treatment was subdivided into grazing paddocks where livestock graze for 1 day out of 40 on any given paddock.

The F treatment is a tropical semi-arid forest that is over 100 years old and was included in the study as an ecosystem control. The tree canopy, over 30 m tall, is composed mainly of *Anacardium excelsum, Xylopia ligustrifolia, Ficus guianensis, Guarea trichllloides*, and *Ceiba pentandra*. The SS treatments were initiated from 1993 to 2005 and are composed of a three-stratum canopy, a herbaceous forage at ground level, shrubs, and tree species (Molina et al. 2001). The SS_3 and SS_8 stands have a bottom stratum mix of *Cynodon plectostachyus* and *Panicum maximum* var. *Tanzania* and *Panicum maximum* var. *Mombasa* grasses, while the SS_{12} has a bottom stratum composed of *C. plectostachyus*. The *P. maximum* var. *Tanzania* and *P. maximum* var. *Mombasa* are grasses with low protein content which were introduced in 1998 to improve the protein-to-energy ratio (Mahecha et al. 2008). The next level is an understory of 10,000 *Leucaena leucocephala* shrubs per hectare that was planted. These shrubs provide high nutritional fodder for cattle and are adaptable to browsing (Kakengi et al. 2001). The upper canopy is of native trees that were left within the SS at densities of 15 to 20 trees ha^{-1} of *Prosopis juliflora* and are more than 15 years old. Other species in SS include: *Albizzia saman, Pithecellobium dulce, Guazuma ulmifolia, Enterolobium cyclocarpum* and palms, *Syagrus sancona*, and *Attalea butyracea* along with *Roystonea regia* and *Chlorophora tinctoria*. The SS_3 paddocks are used for grazing calves at five to seven heads per hectare. The SS_8 and SS_{12} are used for grazing adult dairy cattle at four to five heads per hectare. The combination of grasses and trees ensures a year-round supply of fodder.

The CP has been an intensely grazed native grass monoculture (*C. plectostachyus*) for the past 35 years and receives chemical fertilizers (50 NPK mix kg ha^{-1} yr^{-1}) and herbicides that were applied once a year (March) during the rainy season. Manure was applied twice a year (100 kg ha^{-1}

yr^{-1}). Table 1 shows the general characteristics of the SS chronosequence, CP, and F treatments.

Within each treatment site, two sub-sampling plots (2.5× 2.5 m) were randomly chosen. Soil samples were taken in May 2008. Within each sub-sampling plot 25 (0–10-cm-deep soil cores) were taken with a 2.5-cm diameter probe and then composited. For the F sites, the litter layer was removed prior to sampling. Soil samples were sieved in the field to pass 2 mm, placed in plastic bags, and put on ice. They were stored at −20°C after arrival at the laboratory.

Soil analyses

Soil pH was determined in a 1:1 soil/water (*v/v*) ratio (EPA 2004). Coarse, fine, and total sand, silt, and clay contents were determined by the Bouyoucos hydrometer method (Bouyoucos 1962) and available P by the Bray II extraction method (Bray and Kurtz 1945). Total C was measured by dry combustion at 1,250°C using LECO 2000 CN elemental analyzer (Leco Corp., St. Joseph, MI, USA), and water content was determined gravimetrically after the soil was oven-dried for 24 h at 105°C. Penetration resistance was measured using a STIBOKA penetrometer (Eijkelkamp 1989) as indicator of soil compaction (Martínez and Zinck 2004). Total soil N was determined by Kjeldahl analysis (Bremner and Breitenbeck 1983) and the cation exchange capacity and soil bulk density were evaluated by IGAC (2006).

Acid (AcP) and alkaline phosphatase (AlP) and β-glucosidase (Glu) activities were determined on 1 g of soil and incubated with their buffered substrates (*p*-nitrophenyl phosphate and *p*-nitrophenyl-β-D-glucoside, respectively) at 37°C for 1 h (Dick et al. 1996). The product *p*-nitrophenol (ρNP) was quantified colorimetrically at 410 nm. Urease (Ure) activity was assayed according to Kandeler and Gerber (1988) where 1 g of soil was incubated with a buffered urea solution and the product (ammonium) was measured colorimetrically (660 nm). All enzyme activity assays had two controls: buffered substrate solution without soil and soil without buffer solution but no substrate. Two analytical replicates and one control were analyzed for each soil sample. To standardize the enzyme activity data across sites, results were expressed on per unit C content (μg pNP g^{-1} soil h^{-1} C^{-1}) with enzyme ratios abbreviated as AcP$_C$, AlP$_C$, Glu$_C$, and Ure$_C$ or Glu$_{Cl}$ on organic carbon content basis.

Microbial biomass was determined as the total ester-linked fatty acid methylated esters (FAME) as outlined by Schutter and Dick (2002). In brief, after extraction, FAME were measured on a Hewlett-Packard 5890 Series II gas chromatograph equipped with a HP Ultra 2 capillary column and a flame ionization detector. The viable microbial biomass was calculated by summing FAME concentrations (FAME$_{Tot}$) and reported as nanomoles of FAME per gram of soil (Frostegård et al. 1991; Frostegård and Bååth 1996; Zelles 1997; Feng and Simpson 2009). FAME$_{Tot}$ as an index for microbial biomass was transformed on a C basis and designated as FAME$_C$ (Moore-Kucera and Dick 2008).

Statistical analysis

Results of soil chemical, microbial biomass, and enzyme activities were tested for normal distribution (Shapiro Wilk test) prior to statistical analysis. The land management effect (SS, CP, and F) and effect of chronosequence SS on each enzyme activity were tested using the non-parametric Kruskal–Wallis test and Mann–Whitney *U* tests ($P \leq 0.05$). Analysis of variance was done to determine land management treatment effects on soil physico-chemical and microbial biomass. The Tukey multiple-range test was used for means separation. All statistical analyses were done with SPSS software for Windows (Version 15.0 Inc., Chicago, IL, USA).

Results and discussion

Physicochemical properties

Soil organic matter and total C were significantly higher ($P \leq 0.05$) for CP over other management systems (Table 1). Higher values of soil penetration resistance and bulk density occurred for CP than any other land management systems ($P \leq 0.05$). The F treatment showed the highest values for available P and pH values. In addition, the relative proportions of sand, clay, and silt in our chronosequence of SS and F were similar. However, these were significantly different from CP, especially for clay and sand content (Table 1). Since texture (i.e., clay content) varied between the treatments (SS, F, and CP), we assumed that this was due to inherent differences in the soil and not due to management since there has never been any physical anthropogenic disturbance at these sites and texture generally only changes over geologic time periods.

Clay content was significantly correlated with total C (data not shown) which is consistent with the widely recognized understanding that organic matter increases with clay content (Adu and Oades 1978; Baldock and Skjemstad 2000). Soil organic matter is physically protected against biodegradation through its adsorption on clay and silt surfaces (Hassink 1992; Plante et al. 2006; Grandy et al. 2007). These results support the rationale for normalizing biological or biochemical data on clay or C content (Boerner et al. 2005; Allison and Jastrow 2006; Grandy et al. 2007). The standardized data greatly increased the

Table 1 Physical and chemical properties of soils under different land management

Land use	Age (years)	Texture class	Sand	Silt	Clay	Total C	C clay^{-1} ×100	Total N	OM	BD	SPR	CEC	pH	Available P
			%	%	%	%	%	%	%	g/cc^3	MPa	meg/100g soil		mg kg^{-1}
SS$_{12}$	12–15	Loam	35a	40a	25a	2.51a	10.0a	0.20a	4.42a	1.39b	3.30b	15a	7.15a	74.7a
SS$_8$	8–10	Clay loam	30a	42a	28a	2.38a	8.5a	0.22a	4.30a	1.40b	2.47b	17a	7.20a	100.7a
SS$_3$	3–6	Clay loam	28a	41a	31a	2.60a	8.6a	0.23a	5.41a	1.44b	2.85b	19a	7.16a	67.8a
CP	>30	Silty clay	10b	50a	40b	3.18b	7.9a	0.21a	6.34b	1.52a	3.98a	22a	7.22a	97.9a
F	>100	Clay Loam	29a	37a	34a	2.85b	8.3a	0.20a	5.07a	1.21c	1.49c	17a	8.06b	144.7b

Data followed by same letter within each column did not differ significantly at $P<0.05$

OM organic matter, *CEC* cation exchange capacity, *SPR* soil penetration resistance, *BD* bulk density

sensitivity for detecting land management effects on enzyme activities and microbial biomass. The outcomes of these data, as discussed below, are in agreement with the physical–chemical data with regards to what would be expected for land management effects on soil microbiological properties.

A challenge for conducting research on management of soils is to isolate treatment effects over the confounding and inherent spatial variability of soil across the treatment sites. This occurs because land managers work at large scales and do not normally implement management practices with a statistically valid design. Although we had true landscape replication, Table 1 suggests that soil properties varied as a function of land management because soil particle distributions were different across the treatment sites.

The pH and contents of total C, soil bulk density, and available P varied with land treatment ($P<0.05$). Total C was actually highest for the CP treatment which, based on conventional interpretations, would indicate that this treatment had the highest soil quality. However, when calculated on a per-unit-clay basis, it had the lowest C levels (Table 1). Indeed there was a strong trend that land management was having an effect on organic C/clay ratios with a probability level of $P<0.18$. This would suggest that CP is either losing organic matter or SS systems are accumulating C. This is consistent with the finding that CP treatment had the highest penetrometer readings and soil bulk density; these results suggest that the CP soil is a less favorable medium for root exploration of nutrients and water.

Conversely, the F treatment had substantially lower penetrometer resistance and bulk density, with the SS chronosequence soils being intermediate between F and CP treatments. The higher resistance or inferred compaction in the SS and CP over the F treatment is likely due to the livestock that are not present in the F treatments. The high

plant species diversity of SS, which include woody plant rhizospheres, could resist compaction over the CP with only the rhizosphere of grasses. The lower resistance readings of SS indicate improved aggregation and greater pore space that enhances microbial habitats and activity (Gupta and Germida 1988; Schutter and Dick 2002).

Enzyme activities and microbial biomass

Enzyme activity rarely showed significant differences among the treatments (data not shown). However, normalizing the data on clay content did result in treatment effects that were consistent with management practices and associated C inputs. As an example that is representative of all the microbiological data, Fig. 1a shows Glu$_{Cl}$, where SS$_{12}$ was significantly higher than all the other treatments and the CP had the lowest level of activity.

Data on organic C content basis showed that SS$_{12}$ consistently had the highest enzyme activities (Figs. 1b, 2, and 3), and in some cases SS$_8$ was equal to SS$_{12}$ ($P\leq0.05$). Also, SS$_{12}$ and F treatments were significantly higher than the other SS for Glu$_C$ (Fig. 1). For AlP$_C$, SS$_{12}$ and SS$_8$ were both significantly higher than all other treatments (Fig. 2a), whereas AcP$_C$ (Fig. 2b) and Ure$_C$ activities had stepwise increases with SS age (Fig. 3a). The CP and SS$_3$ treatments consistently had the lowest enzyme activity/C ratios.

As with enzyme activities, FAME$_{Tot}$ was not significantly affected by land management (data not shown). However, we found SS$_{12}$ to be significantly higher than the other treatment ($P<0.05$) FAME on per-unit-clay basis (data not shown) and on per-organic-C basis (Fig. 3b). There were no significant differences ($P<0.05$) for FAME$_C$ among the other land management treatments. Therefore, FAME$_C$ for the SS$_{12}$ treatment was significantly higher in the CP and F systems, which is consistent with the enzyme
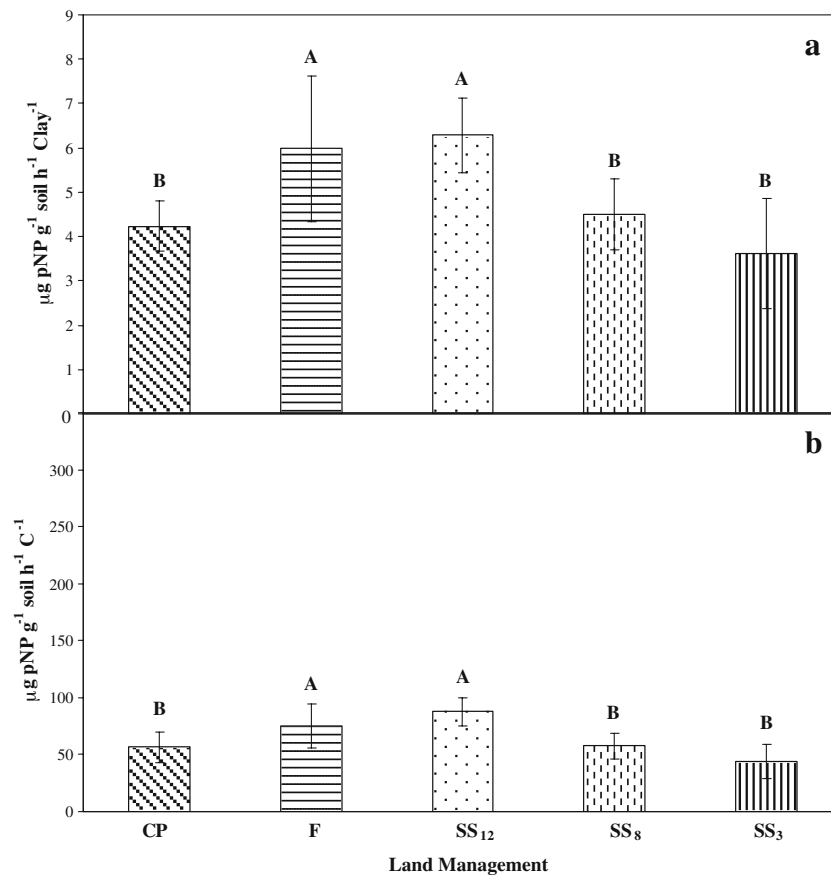
Fig. 1 Ratios β-glucosidase activity per unit of clay (**a**) and per unit C (**b**) as affected by land management. Values with the same *uppercase letters* are not significantly different at *P*≤0.05 among all land management

activity data. The larger microbial biomass of SS_{12} would be expected to produce more enzymes (Asuming-Brempong et al. 2008; Chaer et al. 2009). The low microbial biomass in the CP is likely due to lower C inputs, less plant species diversity, and greater compaction (as shown by penetrometer resistance and soil bulk density) (Udawatta et al. 2009). The high input and diversity of organic materials in the SS is consistent with other studies where microbial biomass is greater under vegetatively diverse systems than in mono-cropped systems (Miller and Dick 1995; Burket and Dick 1998; Klose and Tabatabai 1999, 2000; Ekenler and Tabatabai 2003).

Silvopastoral systems Comparing all the microbiological measures shows a fairly consistent outcome with SS_{12} having the highest levels of enzyme activities and microbial biomass when normalized for C or clay content (data not shown). However, there were more nuanced outcomes and conclusions for specific measures relative to the ranking of various land treatments that will be discussed below.

The ALP_C (involved in P cycling) activity was different from the other enzyme activities where SS_8 was slightly higher than in the SS_{12} (Fig. 2a). Although phosphatase

activity is sensitive to pH, this was likely not a factor in our study because all of the pasture systems (SS and CP) were slightly alkaline and were not significantly different in pH.

The microbial responses indicate that SS is improving soils. However, to fully see this effect, it takes at least 8 years and more likely > 12 years to fully realize a significant effect on the microbiological properties at our site. Our chronosequence results are consistent with one of the few SS studies in tropical regions: that of Isaac et al. (2005) who investigated a multi-canopy cocoa SS agroforestry chronosequence (2 , 15, and 25 years old) in West Africa. Similar to our results, they found that it took about 15 years for the SS system to have levels of soil quality equal to those of an adjacent native vegetation system.

Agroforestry systems can provide a succession of plant species over time. This can cause a shift in the chemistry of C inputs and may account for changes in soils beyond the simple interpretation that more time allows greater C inputs. This was the case in our chronosequence where the oldest SS_{12} and SS_8 had *C. plectostachyus* grass in association with *Leucaena*, *Prosopis*, and *Maclura tinctoria*, whereas SS_3 had a combination of *C. plectostachyus*
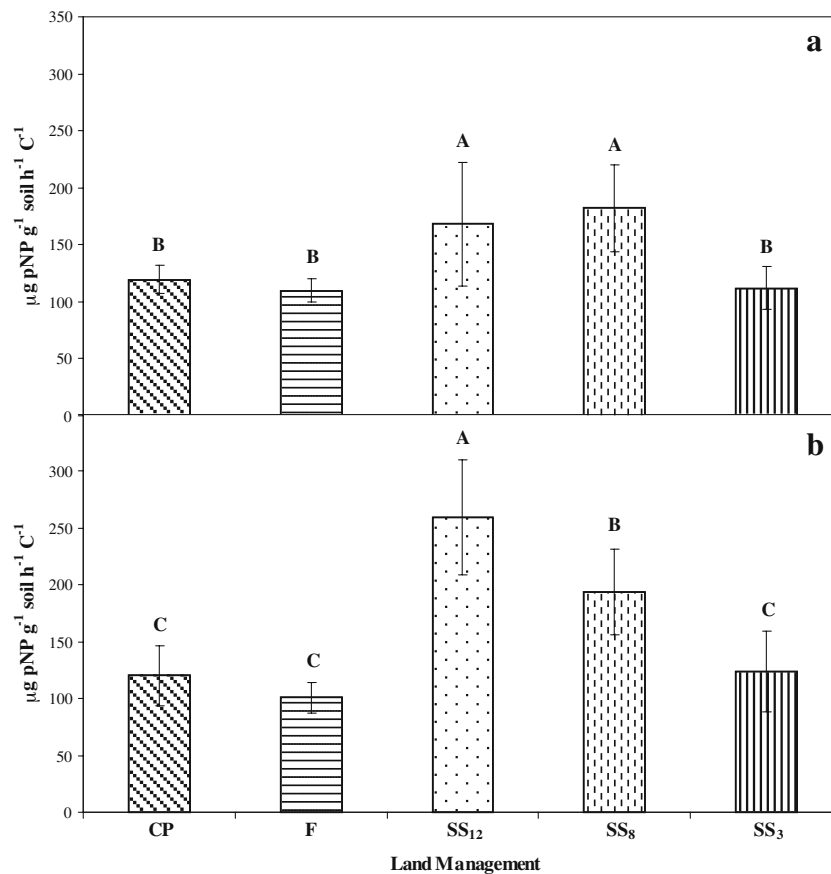
Fig. 2 Ratios alkaline phosphatase activity per unit of C (**a**) and ratios acid phosphatase per unit C (**b**) as affected by land management. Values with the same *uppercase letters* are not significantly different at $P \leq 0.05$ among all land management

and guinea grasses (*P. maximum*) with *Leucaena* and *Prosopis*. Thus, SS_{12} have greater C inputs from woody species, compared to the SS_3 site, which was dominated by herbaceous plants (Tanzania and Mombaza varieties) that are known to have high lignin content (Bot and Benites 2005). This difference in litter chemistry of the C inputs would be expected to shift the microbial community composition and therefore the soil metabolic capacity (Galicia and García-Oliva 2004). This is consistent with Badiane et al. (2001) who reported a shift in plant species from herbaceous to woody species after 11 to 21 years of fallowing and a corresponding significant increase in β-glucosidase and amylase activities.

There are a number of factors that explain the greater microbial responses of SS over CP. One is that, compared to CP, the SS treatments have a much more diverse plant community with a layered canopy. Other studies done at the same sites (Hatico-Colombia) confirmed that there is greater production and quality of fodder biomass in an intensive SS (*L. leucocephala–C. plectostachyus–P. juli-flora*) compared to a CP monoculture (*C. plectostachyus*). Here, fodder biomass measurements were found to be 39 Mg ha^{-1} year^{-1} for an established SS vs. CP, which

provided 23 Mg ha^{-1} year^{-1} (Molina et al. 2001; Mahecha 2003). Thus, SS has greater potential to capture more C in the aboveground biomass than CP. Although we have no belowground measurements, it would be expected that the associated root systems of the multi-species SS plant community have greater belowground biomass that more fully explores the soil profile. This increased above- and belowground C inputs of SS_{12} over CP likely support and drive the microbial responses that we found in this study.

Higher enzyme activities in SS_{12} and SS_8 can be due to greater production of extracellular enzymes from viable cells or to greater stabilization of enzymes in the soil matrix (the abiontic form) (Skujins 1978; Klose and Tabatabai 1999; Acosta-Martinez et al. 2004). In the case of β-glucosidase activity, it may well be that the abiontic form is the most important enzyme fraction for detecting land management effects. This is based on the report of Knight and Dick (2004) who denatured extracellular enzymes associated with viable cells by irradiation and found that the difference in β-glucosidase activity between land management systems on the same soil type was from the abiontic activity and not the activity from viable cells. Conversely, they found that the differences in arylsulfatase
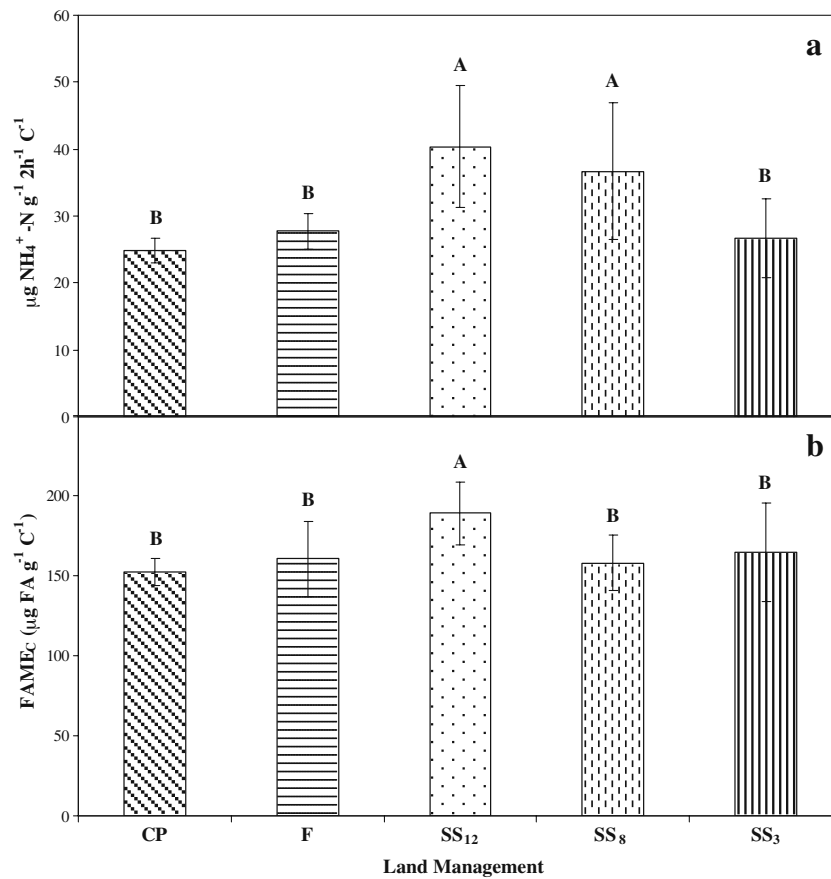
Fig. 3 Ratios urease activity per unit of C (**a**) and ratios FAME$_C$ (**b**) as affected by land management. Values with the same *uppercase letters* are not significantly different at $P \leq 0.05$ among all land management

activity due to land management were caused by activity from viable cells, not from the stabilized enzyme fraction.

The SS$_3$ treatment generally was similar to CP in terms of microbiological activity and total C; this of course suggests that more time is needed to support a larger and more active microbial community. Another factor is that SS$_{12}$ and SS$_8$ are grazed by mature dairy cattle, whereas SS$_3$ had calves feeding on the vegetation. This would change the amounts and chemistry of the manure inputs (Christopher et al. 2003). Calves are more selective grazers than adult dairy cattle and, in turn, this changes the manure and plant root and residue chemistry of C inputs to soils.

Conventional pasture The CP system had lower enzyme activities per unit C and lower FAME$_C$ than SS$_{12}$. Besides the low C inputs of CP, its lack of plant diversity and woody species likely makes it less resilient to compaction from livestock grazing (this is supported by penetrometer data). In turn, compaction would be expected to negatively impact soil microbiological properties by degrading microbial habitat due to poor aggregation (Gupta and Germida 1988; Udawatta et al. 2008).

Another management component specific to CP is that it had the highest inorganic fertilizer inputs. Long-term application of fertilizer inputs can increase (Ghoshal and Singh 1995; Mahmood et al. 1997) or decrease microbial biomass (Smolander et al. 1994; Burket and Dick 1998). However, a factor for enzyme activity suppression by fertilizers is whether it contains the product of a given enzyme reaction. For example, Dick et al. (1988) and McCarty et al. (1992) showed that urease activity was suppressed by long-term NH$_4^+$-based fertilizers, which occurs because repeated applications of the reaction end product, NH$_4^+$, causes feedback inhibition. This would be consistent with our study as CP had lower urease activity than the other treatments. Phosphorus fertilization has been shown to suppress phosphatase activity in agricultural systems (Mathur and Rayment 1977; Nannipieri et al. 1978; Li et al. 2007) and forest systems (Clarholm 1993). Phosphate may inhibit the synthesis of microbial phosphatases and orthophosphate is a competitive inhibitor of acid and alkaline phosphatase activity (Juma and Tabatabai 1978). This could be an additional reason for CP to have low levels of AcP$_C$ and AlP$_C$ activity because repeated

applications of P fertilizer provided the end product, orthophosphate.

Native forest system A curious outcome was that the F system did not have the highest enzyme activities and FAME$_{tot}$ per unit clay (data not shown) or C and indeed had significantly lower levels than did the SS$_{12}$ and was similar to the CP in some measures. This would suggest that the SS system is mimicking the F system relative to soil responses because SS has a similar multistrata canopy and high organic C inputs. Another factor was that the F treatment did not have livestock. Grazing livestock can further affect how a particular vegetative system affects soils. Livestock selectively remove plant material and, given that plant species varied across our treatments, even with the SS chronosequence, this would affect the type of plant material that is released to soils and the chemistry of the animal excreta. Indeed, this was shown by (Patra et al. 2006) where the type of grazing animal affected certain soil community members and enzyme activities. Furthermore, there is evidence that plant litter quality and decomposability increase on grazed plants (Bardgett et al. 1999; Bardgett et al. 2001). Animal manure and urine provide readily utilizable substrates and inputs to soils that influence microbial activities (Clegg et al. 2006).

Soil quality indicators

This study provided an opportunity to compare biological and chemical properties as soil quality indicators that could be used by land managers. Chemical properties including C- to-clay ratios had either no or low sensitivity for detecting land management effects. In contrast, the microbiological properties effectively discriminated between land management systems, independent of soil type, when normalized for soil organic C or clay content. Microbial biomass as measured by total fatty acid concentrations detected management effects of SS after 12 or more years but otherwise showed smaller mean differences and no statistical differences among the remaining treatments.

When we did normalization of the enzyme activity/clay or C ratios and compared the trends and statistical significance to enzyme activities ratios, it appeared that enzyme activities are a precursor of C accumulation. An evidence for this is that SS$_{12}$ had the highest C/clay ratio (10.0) compared to CP with the lowest ratio (7.9) (despite the fact that it had the highest clay content), which would be expected because of the greater C inputs with the SS$_{12}$. However, this trend of C was only moderately significant at $P<0.18$, whereas enzyme activities were significantly different at a lower probability of $P<0.05$. Therefore, it can be assumed that more time (> 12 years) is needed for

organic C accumulation to effectively discriminate between land management treatments. Other research groups have also concluded that enzyme activities are more sensitive to land management than organic C content (Bergstrom et al. 1998; Geisseler and Horwath 2009).

A priori one would expect the F (high plant diversity/C inputs and undisturbed) and SS$_{12}$ (high plant diversity and C inputs) treatment to have high-quality soil. This is supported by low penetrometer resistance, soil bulk density, and a trend for the C-to-clay ratio to be highest for these two systems. If one assumes that these are the highest quality soils in our study, only β-glucosidase activity reflected this as all other microbial measures had F being significantly lower than SS$_{12}$ and not significantly different than CP. This supports findings in temperate regions where B-glucosidase activity was one of the most sensitive enzyme activities for detecting land management effects (Bandick and Dick 1999; Ndiaye et al. 2000; Knight and Dick 2004). A modifying factor that may have contributed to the performance of the F treatment is that, unlike the CP treatment, it receives no fertilizer. Thus, fertilizer nutrients, especially N, could stimulate photosynthesis in CP that did not occur in the F treatment. Additionally, the presence of livestock in CP and SS systems, by converting plant residues to manure, may provide more favorable substrates for microbial communities that would not occur in the F treatment where plant residues of woody species, dominated by lignin and cellulose, may be less stimulatory to microbial activity.

There was a trend for enzyme activities to increase with the duration of SS management but only after 12 years was there a consistently significant difference ($P<0.05$) over the CP for most microbiological measures. We conclude that SS$_{12}$ had significantly better soil quality than SS treatments of < 12 years or CP. These results suggest that adoption of SS promotes ecosystem sustainability by improving the ability of soils to perform decomposition and nutrient mineralization (as reflected by hydrolytic enzyme activities).

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