

Above- and belowground biomass and
nutrient stocks in monoculture and
agroforestry cacao production systems in
the Alto Beni, Bolivia

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Bolivia



Cacao flower (*Theobroma cacao*) (U.S., 2013)

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Abbreviations

AFS = Agroforestry systems

AGB = aboveground biomass

AGC = aboveground carbon

BAR = fallow land (esp. Barbecho)

C = Carbon

Cmic = microbial Carbon

CO₂ = Carbon dioxide

conv = conventional

e.g. = for example (*exempli gratia*)

GHG = greenhouse gas

h = height

h = hours

m a.s.l. = meters above sea level

MCS = Monoculture systems

N = nitrogen

Nmic = microbial Nitrogen

org = organic

pH = inverse, logarithmic scale of the concentration of hydrogen ions in solution

RC = Root carbon

SAFS = Successional agroforestry system

SOC = soil organic Carbon

SOM = soil organic matter

Units

° = degree of arc

' = minute of arc

S = South (latitude)

W = West (longitude)

cm = centimetre (10^{-2} m)

° C = degree Celsius

g = gram

ha = hectare (10^4 m²)

km = kilometre (10^3 m)

km² = Square kilometre (10^6 m²)

m = meter

Mg = Megagram = tonnes (10^6 g)

mm = millimetre (10^{-3} m)

Pg = Petagram (10^{15} g)

Tg = Teragram (10^{12} g)

t = time in days

yr = year

Summary

There is hardly any place left on earth that can be considered untouched nature. Humans penetrate into all areas of this earth, and may it be through greenhouse gas emissions or other types of air and environmental pollution. Deforestation and the conversion of land to agriculture are processes that accompany the spread of humans on this earth, and which shape the landscapes. In this context, tropical forests are nowadays in the focus of forest clearing and land use change. To maintain or restore the ecosystem functions and biodiversity of tropical forests, alternative agricultural land uses are needed. In order to test alternative production systems, the Research Institute of Organic Agriculture (FiBL) launched the research project "Comparison of cropping systems in the tropics" (<https://systems-comparison.fibl.org/>). In Alto Beni (Bolivia), five different cacao production systems are being tested in a long-term trial with regard to their economic, ecological and social impacts. The farming systems range from monocultures to simple agroforestry systems, each under conventional and organic management, to highly complex successional, multistrata agroforestry systems. The plots were established in 2008, in a completely randomized block design, with four replications.

The general objective of this dissertation is to compare, within the long-term experiment in Bolivia, the different cacao cropping systems in terms of their capacity to store and convert carbon, and to draw conclusions on the availability of nutrients through microbial activity. It was hypothesized that (1) AFS store more above and below ground biomass, in the form of carbon, over time, and that (2) both biological management and AFS result in higher biological activity.

To verify this, (1) the different aboveground biomass pools were studied, (2) the biomass obtained from pruning was measured, (3) the annual leaf fall was recorded, (4) the decomposition of leaf litter within one year was analyzed, and (5) the root growth was estimated.

The work performed showed that total aboveground biomass is greater in AFS than in monocultures. However, in the monocultures, the biomass of cacao trees is larger than in the other cropping systems. The total aboveground biomass in AFS is only about one-third of the biomass stored in trees in the surrounding forests. In managed AFS, the

biomass produced by pruning can be twice that of natural leaf fall, and is thus an important source of carbon and nitrogen. The half-life of litter decomposition in the different systems did not differ, despite different microclimates and higher microbial activity in the organically managed plots. Nitrogen-rich leaves of legumes were decomposed faster than lignin-rich cacao leaves. Soil quality is improved 6 years after installation, in the organically managed plots compared to the conventional plots, as evidenced by higher carbon and nitrogen levels, as well as higher microbial activity. Fine root growth is also greater in AFS and biologically managed plots than in the monocultures.

The different studies show that AFS have a pronounced advantage over monocultures in terms of biomass accumulation, even if they do not reach the level of primary or secondary forests. The work shows that there is a strong linkage of the different carbon pools in AFS. More aboveground biomass and fast-growing legumes allow regular pruning, which stimulates carbon and nitrogen cycling. Accumulated litter is decomposed by microorganisms, leading to better soil conditions and nutrient availability.

Therefore, it can be concluded from the present work that AFS cannot per se prevent the clearing of rainforests for agricultural land. However, AFS, unlike monocultures, have a better ecological balance, with more biomass and better soils. The more stable and sustainable AFS are therefore preferable from an ecological perspective to monocultures which are designed for short-term profit.

Zusammenfassung

Auf der Erde gibt es kaum noch einen Ort, der als Unberührte Natur gelten kann. Der Mensch dringt in alle Bereich dieser Erde vor, und sei es durch Treibhausgasemissionen oder andere Arten der Luft- und Umweltverschmutzung. Waldrodungen und die Nutzbarmachung der Flächen für die Landwirtschaft sind Prozesse, die die Ausbreitung des Menschen auf dieser Erde begleiten, und die die Landschaften gestalten. Die tropischen Wälder stehen dabei heutzutage im Fokus der Waldrodung und des Landnutzungswandels. Um die ökosystemaren Funktionen und die Biodiversität der tropischen Wälder zu erhalten oder wiederherzustellen, bedarf es alternativer Landwirtschaftlichernutzungsformen. Um alternative Produktionssysteme zu testen, wurde vom Forschungsinstitut für Ökologischen Landbau (FiBL) das Forschungsprojekte "Vergleich von Anbausystemen in den Tropen" ins Leben gerufen (<https://systems-comparison.fibl.org/>). Im Alto Beni (Bolivien) werden in einem Langzeitversuch fünf verschiedene Kakaoanbausysteme hinsichtlich ihrer ökonomischen, ökologischen und sozialen Auswirkungen getestet. Die Anbausysteme reichen von Monokulturen über einfache Agroforstsystemen, jeweils in konventioneller und ökologischer Bewirtschaftung, hin zu hoch komplexen sukzessionalen, multistrato Agroforstsystemen. Die Flächen wurden 2008, in einem vollständig randomisierten Blockdesign, mit vierfacher Wiederholung eingerichtet.

Das generelle Ziel dieser Doktorarbeit ist im Rahmen des Langzeitversuches in Bolivien die verschiedenen Kakaoanbausysteme zu vergleichen, hinsichtlich Ihrer Kapazität Kohlenstoff zu speichern und umzusetzen, sowie Rückschlüsse zu ziehen auf die Verfügbarkeit von Nährstoffen durch die mikrobielle Aktivität. Es wurden die Hypothesen aufgestellt, dass (1) AFS mehr Ober- und Unterirdischebiomasse, in Form von Kohlenstoff, über die Zeit speichern, und dass (2) sowohl ein biologisches Management als auch AFS dazuführen, dass eine höhere biologische Aktivität vorherrscht.

Um dies zu überprüfen, wurden (1) die verschiedenen oberirdischen Biomassepools untersucht, (2) die anfallende Biomasse durch den Baumschnitt erfasst, (3) der jährliche Blattfall aufgenommen, (4) die Zersetzung der Blattstreu binnen eines jahres analysiert, und (5) das Wurzelwachstum abgeschätzt.

Die durchgeführten Arbeiten haben gezeigt, dass die gesamte oberirdische Biomasse in den AFS größer ist als in den Monokulturen. Jedoch ist in den Monokulturen die Biomasse der Kakaobäume größer als in den anderen Anbausystemen. Die gesamte oberirdische Biomasse in den AFS beträgt nur etwa ein Drittel der Biomasse, die in den Bäumen der umliegenden Wälder gespeichert ist. In gemanagten AFS kann die Biomasse, die durch den Baumschnitt anfällt, doppelt so hoch sein wie der natürliche Laubfall, und ist somit eine wichtige Kohlenstoff und Stickstoff Quelle. Die Halbwertszeit der Streuzersetzung in den verschiedenen Systemen unterschied sich nicht, trotz unterschiedlichem Mikroklima und höherer Mikrobielleraktivität in den organisch gemanagten Flächen. Stickstoffreiche Blätter von Leguminosen wurden schneller zersetzt als die ligninhaltigen Kakaoblätter. Die Bodenqualität ist 6 Jahren nach der Installation, in den biologisch gemanagten Flächen verbessert im Vergleich zu den konventionellen Flächen, was sich sowohl durch höhere Kohlenstoff und Stickstoff Werte bemerkbar macht, als auch in einer höheren mikrobiellen Aktivität. Auch das Feinwurzelwachstum ist in AFS und biologisch gemanagten Flächen größer, als in den Monokulturen.

Die unterschiedlichen Untersuchungen zeigen, dass AFS hinsichtlich der Biomassenakkumulation einen ausgeprägten Vorteil haben gegenüber Monokulturen, auch wenn sie nicht das Niveau der Primär- oder Sekundärwälder erreichen. Die Arbeit zeigt, dass es in den AFS eine starke Verknüpfung der verschiedenen Kohlenstoffpools gibt. Mehr oberirdische Biomasse und schnell-wachsende Leguminosen ermöglichen einen regelmäßigen Baumschnitt, der den Kohlenstoff- und Stickstoffkreislauf anregt. Die anfallende Streu wird von Mikroorganismen zersetzt und führt zu besseren Bodenverhältnissen und der Verfügbarkeit von Nährstoffen.

Aus der vorliegenden Arbeit lässt sich daher ableiten, dass AFS nicht per se die Abrodung der Regenwälder für landwirtschaftliche Flächen verhindern können. AFS im Gegensatz zu Monokulturen jedoch eine bessere ökologische Bilanz aufweisen, mit mehr Biomasse und besseren Böden. Die stabileren und nachhaltigeren AFS sind daher aus ökologischer Perspektive den auf kurzfristigen Gewinn ausgelegten Monokulturen vorzuziehen.

Resumen

Prácticamente no queda ningún lugar en el planeta que pueda considerarse como naturaleza intacta. Los humanos penetran en todas las áreas de esta tierra, ya sea a través de las emisiones de gases de efecto invernadero o de otros tipos de contaminación atmosférica y ambiental. La deforestación y la conversión del suelo a la agricultura son procesos que acompañan a la expansión de los humanos en esta tierra, y que dan forma a los paisajes. En este contexto, los bosques tropicales están hoy en día en el punto de mira de la deforestación y el cambio de uso del suelo. Para mantener o restaurar las funciones del ecosistema y la biodiversidad de los bosques tropicales, se necesitan usos alternativos del suelo agrícola. Para examinar sistemas de producción alternativos, el Instituto de Investigación en Agricultura Orgánica (FiBL) puso en marcha el proyecto de investigación "Comparación de sistemas de cultivos en los trópicos" (<https://systems-comparison.fibl.org/>). En Alto Beni (Bolivia) se están probando cinco sistemas diferentes de producción de cacao en un ensayo a largo plazo con respecto a sus impactos económicos, ecológicos y sociales. Los sistemas de cultivo van desde los monocultivos hasta los sistemas agroforestales simples, cada uno de ellos bajo gestión convencional y orgánica, pasando por los sistemas agroforestales multistrata altamente complejos. Las parcelas se establecieron en 2008, en un diseño de bloques completamente randomizados, con cuatro réplicas.

El objetivo general de esta tesis es comparar, dentro del experimento a largo plazo en Bolivia, los diferentes sistemas de cultivo de cacao en términos de su capacidad para acumular y convertir el carbono, y sacar conclusiones sobre la disponibilidad de nutrientes a través de la actividad microbiana. Se planteó la hipótesis de que (1) los AFS almacenan más biomasa por encima y por debajo del suelo, en forma de carbono, a lo largo del tiempo, y que (2) tanto el manejo orgánico como los AFS dan lugar a una mayor actividad biológica.

Para verificar esto, (1) se estudiaron los diferentes reservorios de biomasa sobre el suelo, (2) se midió la biomasa obtenida de la poda, (3) se registró la caída anual de hojas, (4) se analizó la descomposición de la hojarasca en un año, y (5) se estimó el crecimiento de las raíces.

El trabajo realizado mostró que la biomasa total sobre el suelo es mayor en los AFS que en los monocultivos. Sin embargo, en los monocultivos, la biomasa de los árboles de cacao es mayor que en los otros sistemas de cultivo. La biomasa total sobre el suelo en AFS es sólo un tercio de la biomasa acumulada en los árboles de los bosques circundantes. En los AFS bien manejados, la biomasa producida por la poda puede ser el doble de la caída natural de las hojas, y es por lo tanto una importante fuente de carbono y nitrógeno. La vida media de la descomposición de la hojarasca en los distintos sistemas no difirió, a pesar de los diferentes microclimas y de la mayor actividad microbiana en las parcelas manejadas orgánicamente. Las hojas ricas en nitrógeno de las leguminosas se descompusieron más rápidamente que las hojas de cacao, ricas en lignina. La calidad del suelo mejoro 6 años después de la instalación, en las parcelas manejadas orgánicamente en comparación con las parcelas convencionales, como lo demuestran los mayores niveles de carbono y nitrógeno, así como una mayor actividad microbiana. El crecimiento de las raíces finas también es mayor en las parcelas de AFS y de gestión biológica que en los monocultivos.

Los diferentes estudios muestran que los AFS tienen una pronunciada ventaja sobre los monocultivos en términos de acumulación de biomasa, aunque no alcancen el nivel de los bosques primarios o secundarios. Los trabajos muestran que existe una fuerte vinculación de los diferentes depósitos de carbono en los AFS. La mayor biomasa sobre el suelo y las leguminosas de rápido crecimiento permiten una poda regular, que estimula el ciclo del carbono y del nitrógeno. La hojarasca acumulada es descompuesta por los microorganismos, lo que permite mejorar las condiciones del suelo y la disponibilidad de nutrientes.

Por lo tanto, del presente trabajo se puede concluir que el AFS no puede impedir per se la deforestación de los bosques tropicales para la obtención de tierras agrícolas. Sin embargo, los AFS, a diferencia de los monocultivos, tienen un mejor balance ecológico, con más biomasa y mejores suelos. Por lo tanto, los AFS, más estables y sostenibles, son preferibles, desde una perspectiva ecológica, a los monocultivos diseñados para obtener beneficios a corto plazo.

1. General introduction

As part of the geosystems, each life form influences and changes the environment in specific ways. Humans as a life form and part of this environment not only influence and change, but also have a transformative effect. Since humans have become sedentary, they have begun to intervene decisively in their environment. Every interaction with the environment by humans has had and continues to have the effect of changing what is commonly referred to as nature. Nature thus stands in contrast to culture, that which is created by humans. As humans are omnipresent on earth, the question arises as to what is still nature or natural. In their study from 2021, Plumptre et al. come to the conclusion that only "... 2.8% of the land surface that could be considered functionally intact." The majority of the terrestrial surface is thus directly influenced by humans. This is also reflected in the discussion about the introduction of a new geological age, the Anthropocene by Paul Crutzen in 2000 (Steffen et al., 2007). What was considered nature or wilderness has all but disappeared. A truly pristine nature no longer exists. It has been replaced, and what has remained is an understanding of nature that is a human construct. Nature and especially the natural landscape can be unaffected by human activities, but behind it is a human-designed ideal.

Like the concepts of landscape (Hard, 1969; Eisel, 1982; Kirchhoff, 2009; Kühne & Antrop, 2015), which can have different meanings; in geography it is often used to delimit a space with natural scientific categories. But most definitions of landscape, however, are characterized by the fact that "The concept of landscape does not denote a natural scientific entity, but a socio-cultural, primarily aesthetic-symbolic one, and it is inseparably linked to certain ways in which people interpret their environment. (Kirchhoff, 2011)". Landscape as a construct with aesthetic properties and cultural values is thus to be distinguished from ecosystems, with biotic and abiotic units (Kirchhoff, 2013).

This dissertation in the area of landscape ecology and geography deals therefore, on the one hand, with a specific landscape and its elements, which includes its socio-cultural and aesthetic characteristics. Specifically, it is about different cacao production systems in the agricultural landscape of the Alto Beni, Bolivia. On the other hand, with an ecological perspective on a particular (agro-)ecosystem. In detail, with the ecological

characteristics of monoculture (MCS) and agroforestry systems (AFS) under conv and org management. The general objective of the dissertation is the quantification of carbon (C) and nitrogen (N) stocks, the potential to reduce C-emissions and the improvement in cacao production systems (monoculture or agroforestry), which are conventionally or organically managed.

In the following, this introductory chapter will focus on the different ecological aspects related to the challenges of cacao agroecosystems.

1.1. Deforestation and land use change in the tropics

Forests cover 4.06 billion hectares worldwide, 1.8 billion hectares are located in the tropics, representing nearly half of the total coverage (45%) (FAO, 2020). Carbon, which is stored in tropical forest vegetation, is estimated to range from 228.7 (Baccini et al., 2012) to 247 Pg C (Saatchi et al., 2011). While Pan et al. (2011) give a total C stock (above and below ground) of about 471 Pg C.

The tropical and subtropical forests have therefore a special position concerning climate change and greenhouse gas (GHG) emissions. The conversion of tropical forests into agriculture land is triggering loss of biodiversity and contributes significantly to increase GHG concentrations and thus intensifies climate change (Edwards, 2019; Seymour and Harris, 2019). Forests and especially tropical forests play a significant role in terms of regional and global climate regulation and they are important C sinks. Deforestation contributes significantly to the reduction of this C sink and to the emission of atmospheric GHG. Furthermore, forests provide a multitude of ecosystem services including food and timber products, C storage and watershed and soil protection. The unsustainable conversion and use of tropical forest land can lead to soil degradation (Nair et al., 2009; FAO, 2017; Veldkamp et al., 2020, van Noordwijk 2021) and associated socio-economic problems (Jacobi, 2016; Montagnini and Metzger, 2017).

The second largest anthropogenic source of C emission globally is deforestation (van der Werf et al., 2009). The share of deforestation and forest degradation on anthropogenic CO₂ emissions are estimated between 6 and 20% (van der Werf et al., 2009, Saatchi et al., 2011, Houghton et al., 2012). Harris et al. (2012) estimate that Latin America's contribution to total emissions from deforestation is up to 54%. Latin America is

therefore the greatest contributor of CO₂ emissions from forest conversion to agricultural land (Carter et al., 2017). In Latin America, 78% of forest conversion is the result of agricultural activities. In the period from 2000 to 2005 the CO₂ emissions in Latin America due to agriculture-driven deforestation reached 974±148 Tg yr⁻¹ (Carter et al., 2017). For Bolivia the gross forest cover loss from 2000 to 2005 is calculated at 1290 km²yr⁻¹. This sums up to a C loss per year by rainforest deforestation of 11 Tg C yr⁻¹ or 40.37 Tg CO₂ yr⁻¹ (Harris et al., 2012).

Using Brazil as an example, Tyukavina et al. (2019) show that agro-industrial clearing for grassland accounts for 63% of total deforestation, while small-scale forest clearing accounts for 12% and agro-industrial clearing for cropland only 9% (Carter et al., 2017). That small scale clearing in the Amazon region is exceeding agro-industrial clearing for cropland can be seen across all of South America (Seymour and Harris, 2019). Smallholder and subsistence agriculture, which usually only clears an area of less than 10 ha, is nevertheless partly responsible for a significant part of deforestation.

According to Putz et al. (2000), conservation of tropical forests, even under the most optimistic scenarios, is not sufficient to maintain biodiversity and ecosystem services.

Developing alternative strategies and finding solutions to reduce deforestation that are compatible with human needs are important issues with regard to increasing GHG emissions. Afforestation and reforestation in the tropics are two options for C sequestration, but are not an alternative for agricultural land use. For the tropics and the subtropics, as well as for the temperate zone, AFS are already considered as feasible land use practices in the current climate debate (Neufeldt et al., 2012). Current research considers AFS as a possible alternative in terms of GHG reduction and C storage (Mutuo et al., 2005; Nair et al., 2009; Saj et al., 2017; Abou Rajab et al., 2016).

1.2. Agroforestry systems (AFS) and Organic Farming

There are a multitude of definitions for AFS, which shows how many ways there are to design and implement AFS (Nair 1993; ICRAF 2000; FAO 2017). Leakey (1996) defined AFS as “...a dynamic, ecologically based, natural resource management system that, through the integration of trees in farm- and rangeland, diversifies and sustains smallholder production for increased social, economic and environmental benefits”

Nair et al. (2009) estimates the global area under AFS to 1023 million ha. The most common AFS in the tropics are annual and perennial crops grown under shade, like coffee plants (*Coffea arabica* L.) and cacao trees (*Theobroma cacao* L.) (Willer and Lernoud, 2019), or multistrata and SAFS. These agricultural systems may differ by retaining existing trees, actively planting trees, and tolerating spontaneous tree growth (Schroth et al., 2004). Trees in AFS can perform a variety of ecological functions and also provide a direct economic benefit for the producers (Udawatta et al., 2017; Barrios et al., 2018; van Noordwijk, 2021). Furthermore, the AFS itself offer a large number of economic benefits for the producers, like income diversification, food security and food sovereignty (Jacobi, 2016; Schneider et al., 2017, Montagnini and Metzler, 2017). In terms of the environment, AFS offer many ecosystem services, like maintaining of biodiversity, buffering climatic oscillations, protecting soil and water resources (Asare, 2006; Jose, 2009; Mortimer et al., 2017; Niether et al., 2018; Tschardt et al., 2011). The high complexity and interaction in the composition of perennial plants, including trees and shrubs and various herbaceous plants, leads to a broader diversity of plant species with different eco-physiological characteristics. This abundance allows for more efficient use of natural resources, especially competition for water, nutrients, light and CO₂, and promotes a more stable and resilient system that can mitigate climate change impacts such as droughts or heavy precipitation events (Rao et al., 1998; Jose, 2009; Nair et al., 2010; Haggard et al., 2011).

Even though, as shown above, AFS can offer ecological and economic benefits, they are part of the (agricultural) landscape, and result from a socio-cultural and aesthetic concept of value. The integration of AFS into the agricultural landscape therefore shows which understanding of the environment prevails. An agricultural landscape, even if it is designed with AFS, is always based on an earlier intervention in nature. This is usually associated with a change in the species community or even leads to a loss of biodiversity. Therefore, despite the benefits, further deforestation should be avoided and the installation of AFS should be limited to open and/or degraded areas (Martin et al., 2020).

Nevertheless, due to ecological and economic benefits AFS are perceived as a land management system that contributes to United Nations Sustainable Development Goals (van Noordwijk et al., 2018). Furthermore, most AFS are designed for low or no input

agriculture by an effective internal nutrient cycle that includes leguminous trees and pruning management. Inorganic, chemical fertilisers can thus be avoided, which in turn reduces the economic dependency of farmers on the agrochemical market (Johns, 1999).

AFS, as described above, can therefore comply with organic agriculture standards without major difficulties, mainly for small-scale farmers. The International Federation of Organic Agriculture Movements (IFOAM) formulates the basic principles of org farming as follows, health, ecology, fairness and care (IFOAM, 2005; Luttikholt, 2007).

Insofar, there are many parallels of AFS and org agriculture, the preservation of biodiversity and soil fertility (Kilcher, 2007), the absence of industrial chemical inputs, and a wide range of products and premium prices that can improve farmers' livelihoods (Armengot et al., 2016).

However, there are also various prejudices and criticisms against AFS and org farming, which are not essentially different. The main point is that both farming systems are not sufficiently productive and finally requires more land to produce the same amount of food (Connor, 2008; Trewavas, 2001). Studies by Schneider et al. (2017) and Seufert and Ramankutty (2017) show that this need not be the case, but that even more and more variable production can be achieved on the same space. Therefore, one of the main objectives of modern agroforestry is the concept of sustainable intensification or eco-intensification to optimise production and use resources in a more sustainable way (Santiago-Freijanes et al., 2018). Lower yield production need not be seen as negative per se if the system is more sustainable.

1.3. Carbon and nutrient stocks, in agroforestry systems

Since the last 20 years, AFS have come into focus for C sequestration (Jose, 2009; Nair et al., 2009). The establishment of an AFS allows the uptake of C by vegetation. Mostly dominated by shade trees, afforestation systems offer the potential to store C above- and belowground, and could thus serve as a sink for GHG (Mutuo et al., 2005; Saj et al., 2013; Monroe et al., 2016). Kessler et al. (2012) conclude that C rich AFS can store about 60% of the C stored in primary forests. Recent studies show that cacao AFS can store up to 140 Mg C ha⁻¹ in the AGB. (Saj et al., 2017). How much C is, or can be, stored in an AFS

depends on many factors. The type and number of trees per hectare, management practices, and age of the plots play an important role in the regional variation of AGB (Abou Rajab et al., 2016; Beer, 1988; Nair et al., 2009). The regional differences are thus also related to climatic and geological conditions and cultural circumstances. Studies of above- and belowground C storage and turnover in afforestation systems have thus faced and continue to face the problem of heterogeneity and defining reference stages (Ekanade et al., 1991; Hartemink, 2005). Biomass production of AFS is also depending on the availability of plant nutrients such as nitrogen (N) and phosphorus (P).

According to Rao et al. (1998), the main biochemical interactions of C and N in AFS are seen between trees, annual plants, and soil. The interaction of all plants in AFS have a positive impact on the quality and quantity of soil organic matter (SOM) by crop and pruning residues, plant litter and root turnover. The availability of plant biomass (organic matter and nutrients) is an important basis for the soil fauna, stimulates it and enhances the microbial matter transformation (Nair et al., 2009). In terms of nitrogen, AFS trees with deep roots stimulate the decomposition of nitrogen stocks and the root net prevents nitrogen leaching (Beer, 1988; Jose, 2009). The inclusion of fast-growing and nitrogen-fixing legumes further increases nitrogen supply as nitrogen is taken up into the plant biomass and returned to the soil via litterfall and pruning (Albrecht and Kandji, 2003; Beer et al., 1990).

1.4. Alto Beni Region and the FiBL long-term trial (SysCom) in Bolivia

The Alto Beni region takes its name from the Alto Beni River (Figure 1). The Alto Beni rises on the eastern slope of the Bolivian Cordillera Real and flows parallel to the Andes in a northerly direction. From the breakthrough through the foothills at Rurrenabaque the river is called Rio Beni. The Rio Beni flows together with the Río Mamoré and becomes the Rio Madeira, the largest affluent of the Amazon.

The Alto Beni region is located about 140 km air-line distance northeast of La Paz, at the transition from the Andean highlands to the lowlands (Ahlfeld, 1972; Gerold, 1987; Montes de Oca, 1989). The transition zone is characterized by several parallel mountain ranges (up to 200 meters) and wide valleys, with the Rio Beni flowing in the main valley. The main valley is between one and five kilometres wide, and lies at a sea level of about 400 metres (Ticona Cuba, 1994; Elbers, 2002).

According to the classification of Troll and Pfaffen (1964), it is a tropical-summer humid climate with a pronounced dry season from mid-April to the end of September and two almost arid months (July and August). The mean annual precipitation is around 1500 mm, and the average monthly air temperature ranges between 22° C in July and 27° C in December (Elbers, 2002; Niether, 2018).

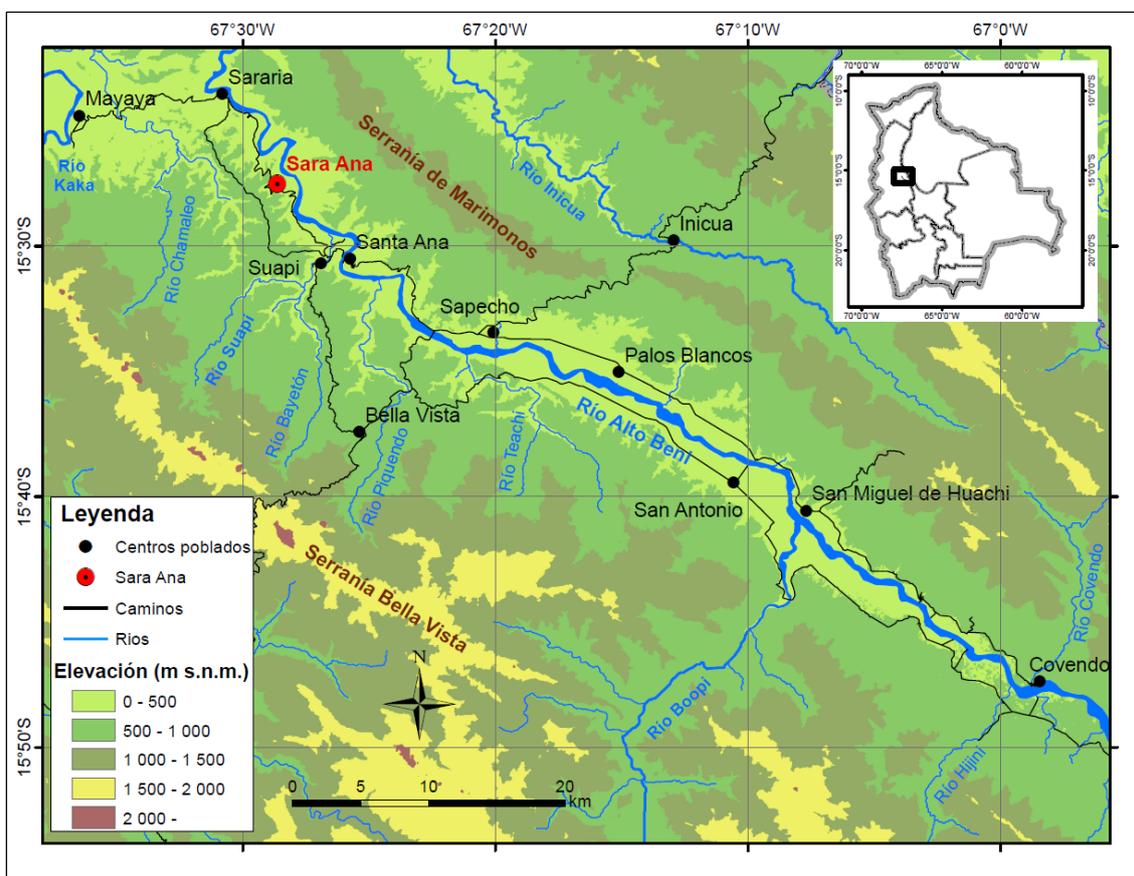


Figure 1: Map of the Alto Beni Region in Bolivia with the SysCom research station Sara Ana (Ripa et al., 2022).

The Rio Beni divides the region into two main administrative districts. The municipio Alto Beni, on the western side of the river as part of the province of Caranavi, and the municipio Palos Blancos, on the eastern side of the river as part of the province Sur Yungas.

The landscape is characterised by the alternation of remnants of a (near-) natural forest and agricultural land. The forest community in Alto Beni is a mainly seasonal evergreen forest composed of species from the Yungas and species from the Amazon basin (Seidel and Vargas, 1994; Ibsch, 2004; Navarro, 2011). In many areas, the species-rich primary forest has been replaced by secondary vegetation. Most of the forest conversion takes

place on the flat and wide river terraces, as these are the preferred areas for the cultivation of agricultural products. In the places where the areas are abandoned, a dense herbaceous and dense herb and shrub layer (*chume*) grows within a very short time. This natural fallow develops to a 15 to 20 m high secondary forest (*barbecho*) within five to seven years.

The relatively easily accessible river terraces of the valley floors are intensively farmed on a large scale. As the slope increases, the intensity of the cultivated land decreases. On the recent river terraces, primarily annual and perennial crops are cultivated like, rice (*Oryza* L.), bananas (*Musa* spp. L.), papaya (*Carica papaya* L.), melons (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), yuca (*Manihot esculenta* Crantz), etc.). In the flood-free areas, the cultivation of cacao (*Theobroma cacao* L.) and citrus fruits (*Citrus* L.) dominates.

On one of these flood-free river terraces of the Alto Beni, in Sara Ana (15°27'S, 67°28'W), northwest of Sapecho and Palos Blancos, a research station and a long-term experiment were established in 2007 (Figure 1). Sara Ana is part of the project "Farming Systems Comparison in the Tropics" (<https://systems-comparison.fibl.org/>), which was set up by the Research Institute of Organic Agriculture (FiBL) in Kenia, India and Bolivia. The aim is to study conv and org production systems in long-term experiments (LTE). In Sara Ana, the project has the objective to study different forms of cacao cultivation (Schneider et al., 2017). Therefore, in 2007, a several years old fallow forest was cleared.

After one year with maize cropping, at the end of 2008, cacao trees for the experimental plots were planted (Schneider et al., 2010). Five different cacao production systems and a fallow plot were arranged in a complete randomised block design with four replicates (Schneider et al., 2017).

The following treatments were defined (Figure 2):

- 1) monoculture, full sun with conventional management
- 2) monoculture, full sun with organic management
- 3) diversified, shaded agroforestry system with conventional management
- 4) diversified, shaded agroforestry system with organic management
- 5) diversified, shaded successional agroforestry system with organic management
- 6) fallow land (no crops) as a reference for biodiversity and soil fertility studies

The treatments increase in complexity and biodiversity: from full sun to AFS to SAFS. The plots of SAFS and fallow land (BAR) treatments add value to the experiment by having references for C sequestration and nutrient cycling under “ideal management” (SAFS) or “no action” (BAR). The org treatments comply with the European regulation for org production. Conventional treatments are based on best practices, adapted to the Bolivian context. Organic management of the plots consists of the application of compost produced on farm and covering the soil with a perennial soybean (*Neonotonia wightii* (Wight and Arn.) J.A. Lackey), for weed control, biomass accumulation and nitrogen fixation. In conv systems mineral fertilizer and herbicides were applied (Schneider et al., 2017). Cacao trees were planted in a distance of 4 by 4 m (625 trees ha⁻¹) as it is usual in the region. Gross trial plots have a size of 48 by 48 m, with a net plot of 24 by 24 m, to avoid border effects. The AFS consist of various tree species and also palms with a spacing of 8 m (227 trees ha⁻¹). In addition to high grade wood and fruit trees, fast-growing leguminous trees (*Erythrina* spp. and *Inga* spp.) were planted for biomass accumulation and as a nitrogen source. Bananas (*Musa × paradisiaca* (L.)) were planted in the AFS as an additional source of income for the first few years (Armengot et al., 2016).

Following best practices, the cacao trees are pruned three times a year. A thinning pruning to improve aeration, light penetration, flowering and fruit set, and a phytosanitary pruning at the end of the harvest season. For the agroforestry trees, the annual pruning is done at the beginning of the rainy season in December. The pruning residues are chopped and spread at a distance of 0.5-1 m around the tree trunk.

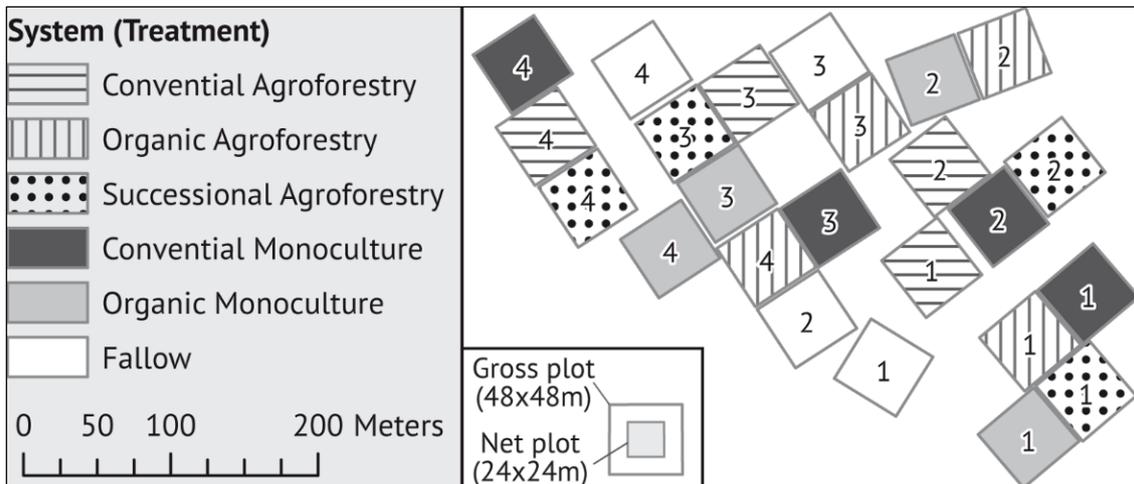


Figure 2: Experimental setup of the FiBL long-term field experiment in Alto Beni, Bolivia. Comparing cacao monoculture systems and agroforestry systems under conventional versus organic management. Numbers in plots represent block (replication), minimum distance between two plots = 2 m (modified after Schneider et al., 2017).

1.5. Aim and objectives of the study

The aim of this study is to compare different agriculture production systems in terms of biomass accumulation and nutrient availability in order to assess the impact of land use and land cover changes, from a landscape ecological perspective. The choice for which agriculture production systems a producer decides, is socio-culturally and politically determined and has an influence on the landscape, and its future. Research and knowledge transfer can influence this decision.

The general question behind this study is about the ecological consequences of change in and into an agricultural landscape, through different production systems. Only when a system is capable to cope with changing conditions, e.g. by a more balanced resource use, it is capable to maintain a high productivity (C gain) and biomass (C storage).

In specific, the question of what impact the installation of a cacao MCS has on the system compared to an AFS has to be investigated. As well as the question of what influence org and conv management has on the system. To address this question, different biomass and nutrient pools of four different cacao production systems are to be quantified.

It is hypothesised that AFS store more biomass, in form of C, in the long-term, both above- and belowground. Furthermore, it is assumed that org managed production systems and AFS show greater soil biological activity and a higher root production.

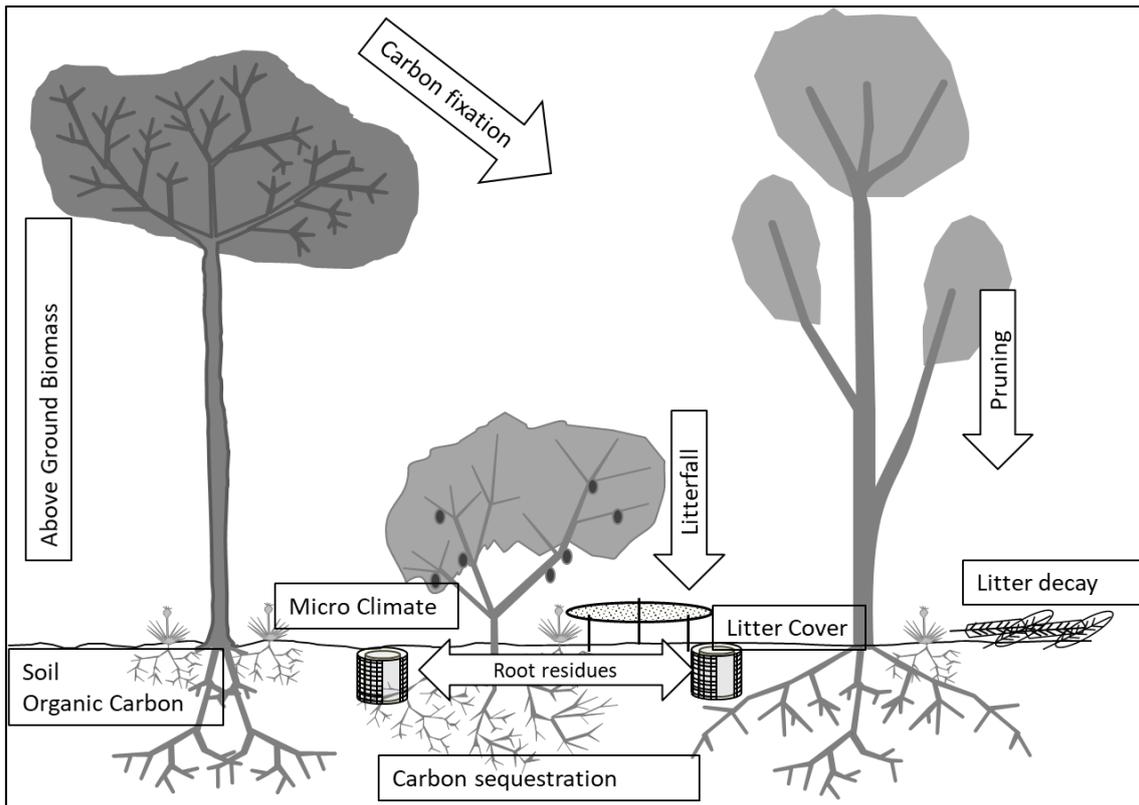


Figure 3: Experimental design with the parameters measured in the field plots.

The specific steps to achieve this objective are (Figure 3):

1. To evaluate the aboveground biomass pools, like plant biomass, litterfall and pruning residues.
2. To measure the SOC and N accumulation and the microbial activity.
3. To quantify the belowground biomass in form of root production.

Due to the specific research objectives, the thesis is divided into three main chapters, which are reflected in the respective publications. The original articles have been slightly modified from the published versions in order to present a more uniform image of the present thesis. In particular, the term cocoa and cacao were unified in this thesis. The term cacao is used for the plant and the production systems in derivation of the scientific classification of the species *Theobroma cacao* (L.). Cocoa is used in relation to the processed (Post-harvest product) beans. Furthermore, the abbreviations for the production systems (AFS = agroforestry system and MCS = monoculture system) were unified.

Chapter 2 contains the study about aboveground biomass pools and the quantification of litterfall and pruning residues, with the following specific research questions:

- 1) How much more can be stored in org cacao production systems compared to conv cacao production systems in both AFS and MCS?
- 2) To what extent do the amounts of litterfall and pruning residues in AFS exceed those of MCS?
- 3) How much increase in N supply is achieved by including N-fixing trees in AFS?

Chapter 3 deals with the increase of soil C and nitrogen levels and the microbial biomass in org managed cacao AFS.

The following research questions were addressed:

- 4) Do SOC and N contents differ with respect to crop diversity and management practices?
- 5) In which cacao production system is microbial biomass highest?
- 6) Does the incorporation of legume trees, such as *Erythrina* spp., in AFS increase the microbial activity?
- 7) Is there an effect on litter decomposition rates due to the cacao production system?

Chapter 4 meets the subject in the context of this study of the belowground root production.

The following hypotheses were tested:

- 8) That the cacao fine roots become smaller and less abundant as the distance from the stem increases.
- 9) That the cacao fine roots in the organically-managed MCS and AFS are more abundant than in the conventionally-managed MCS and AFS.
- 10) A higher total fine root production in AFS due to higher stem density.

2. Carbon stocks, litterfall and pruning residues in monoculture and agroforestry cacao production systems



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Carbon stocks, litterfall and pruning residues in monoculture and agroforestry cacao production systems

2.1. Summary

Agroforestry systems (AFS) can serve to decrease ecosystem carbon (C) losses caused by deforestation and inadequate soil management. Because of their shade tolerance, cacao plants are suitable to be grown in AFS, since they can be combined with other kinds of trees and shrubs. The potential for C sequestration in cacao farming systems depends on various factors, such as management practices, stand structure and plantation age. We compared conventionally and organically managed cacao MCS and AFS in Sara Ana (Bolivia) with respect to C stocks in plant biomass and to amounts of litterfall and pruning residues. The total AGC stocks of the AFS (26Mg C ha^{-1}) considerably exceeded those of the MCS ($\sim 7\text{Mg C ha}^{-1}$), although the biomass of cacao trees was greater in the MCS compared to the AFS. Due to higher tree density, annual litterfall in the AFS ($2.2\text{Mg C ha}^{-1}\text{year}^{-1}$) substantially exceeded that in the MCS ($1.2\text{ Mg C ha}^{-1}\text{year}^{-1}$). The amounts of C in pruning residues ($2.6\text{ Mg C ha}^{-1}\text{year}^{-1}$ in MCS to $4.3\text{ Mg C ha}^{-1}\text{year}^{-1}$ in AFS) was more than twice those in the litterfall. Annual nitrogen (N) inputs to the soil through pruning residues of cacao and N-fixing trees were up to 10 times higher than the N inputs through external fertiliser application. We conclude that appropriate management of cacao AFS, involving the pruning of leguminous trees, will lead to increases in biomass, litter quantity and quality as well as soil C and N stocks. Thus, we recommend stimulating the expansion of well-managed AFS to improve soil fertility and enhance C sequestration in soils.

2.2. Introduction

Global climate change is driven by the anthropogenic increase of atmospheric greenhouse gases such as CO_2 (IPCC, 2014). The second largest anthropogenic source of CO_2 emissions is deforestation (van der Werf et al., 2009). The share of deforestation and forest degradation in total anthropogenic CO_2 emissions is estimated to be approximately 20% (van der Werf et al., 2009). Half of the global CO_2 emissions from deforestation are related to the clearing of tropical forests in Latin America (Harris et al., 2012). For instance, in Bolivia, the gross forest cover loss due slash-and-burn

practices from 2000 to 2005 is estimated to 1290 km² year⁻¹, which corresponds to an annual C loss of 11 Tg C year⁻¹ (Harris et al., 2012). Therefore, the loss of tropical and subtropical forests plays a prominent role in climate change.

Reducing CO₂ emissions by limiting deforestation, and increasing ecosystem C stocks by afforestation and reforestation are possible ways to decrease the quantity of greenhouse gases in the atmosphere. However, another key to preserving C stocks in tropical lowlands is the implementation of sustainable land management alternatives (Albrecht and Kandji, 2003), such as AFS. AFS also mitigate losses of biodiversity, prevent soil degradation and maintain air and water quality (Mortimer et al., 2017; Tschardt et al., 2011). Sufficiently complex AFS, composed of perennial plants, trees and shrubs, and combined with various annual crops, can increase the diversity of plant species with different physiological properties, allowing for a more efficient use of soil resources (Jose, 2009). Various AFS trees and other plants have a positive influence on the quality and quantity of soil organic matter (SOM) through input of crop and pruning residues, litterfall and root turnover. Moreover, through these pathways, organic matter and nutrient inputs stimulate the soil fauna and microbial matter transformations (Nair et al., 2009).

Further advantages of AFS are obtained by (i) including fast-growing and nitrogen (N)-fixing leguminous trees, e.g., *Erythrina* spp. and *Inga* spp. (Albrecht and Kandji, 2003; Beer et al., 1990), since these trees increase the N supply for all AFS plants by incorporating N into their own biomass and contributing it to soil N stocks through litterfall; (ii) the function of deep-rooting trees as nutrient pumps, as they extract nutrients from sub-soils and return them to top-soils through litterfall (Beer, 1988); (iii) reduced N leaching, since AFS, compared to MCS, are characterised by increased biomass production and thus enhanced uptake of nitrate, and deep-rooting AFS trees may still reach nitrate that has been leached to a depth where other plants cannot reach it anymore (Jose, 2009). Due to these effects, AFS allow for a substantial reduction of external inputs of inorganic fertilisers, compared to MCS. In this way, the economic dependency of farmers on the agrochemical market can be reduced (Johns, 1999). This effect is well in-line with the intention of org agriculture that also aims at avoiding chemical inputs and promotes production systems that are adapted to local conditions.

Some advantages of org agriculture include (i) the preservation of biodiversity and soil fertility (Kilcher, 2007), (ii) reduced production costs due to the omission of industrial-chemical inputs and (iii) a wide range of products and premium prices for org cash crops that may enhance the economic living conditions of the farmers (Armengot et al., 2016). Thus, the practices of AFS are compatible with the principles and perspectives of org agriculture, mainly for small-scale farmers.

Cacao (*Theobroma cacao* L.) is a shade-tolerant plant and an important cash crop in tropical countries. Cacao is mostly grown by small-scale farmers, who produce 80–90% of the cacao worldwide (WCF, 2014). In 2016/17, a total of 4.7 million tons of cocoa beans were produced on approximately 10 million ha (ICCO, 2017). Organic production makes up less than 2.5% of total cacao production. Cacao production is predominantly located in South America, where more than 80% of the world's org cacao is produced (Lernoud and Willer, 2016). In 2014, org farming was practised on an estimated area of 6.8 million ha in Latin America (1.1% of the agricultural area). In Bolivia, the percentage of agricultural area that is farmed organically has reached only 0.3% (114 306 ha). A total of 12 114 producers practised org agriculture in 2014 (Lernoud et al., 2016). These data demonstrate that the potential for org agriculture in Latin America, and particularly in Bolivia, is by far not yet realised. However, the above mentioned benefits must be quantified and evaluated for each region and then disseminated to farmers in order to effectively promote org agriculture.

Organically managed cacao AFS, in addition to their beneficial effects on farmers' income and on ecosystem services, offer a high capacity for C sequestration (Saj et al., 2013). In the past, this aspect of C sequestration was not as much in the focus of research as the economic and ecological aspects of AFS, which have been extensively analysed and compared to those of MCS's shifting cultivation (Steffan-Dewenter et al., 2007). Only in the past 20 years has the potential of AFS for C sequestration received increasing attention (Jose, 2009; Nair et al., 2009). Current studies show that cacao AFS may store up to 140 Mg C ha⁻¹ in AGB alone (Saj et al., 2017). The amounts of stored C mainly depend upon the composition of the tree species, tree density and the age of the AFS (Abou Rajab et al., 2016; Beer, 1988; Nair et al., 2009). However, management practices and cacao tree age can vary widely within a region, which makes comparisons difficult.

Therefore, the FiBL (Research Institute of Organic Agriculture, Switzerland) established a systematic long-term field trial in the eastern Andean foothills of Bolivia, where the most common cacao MCS and AFS under org and conv management are compared (<http://www.systems-comparisons.fibl.org>).

Due to the widespread cultivation of cacao as a cash crop, an assessment of the potential impacts of different cacao production systems on C sequestration and N supply is of particular relevance. Therefore, in the present study, we compared different cacao production systems with respect to C stocks in the plant biomass, litter and pruning residues, and to temporal changes in C stocks. Thereby, we focussed on the following research questions: (i) how much more C can be stored in org cacao production systems compared to conv production systems in both AFS and MCS? and (ii) to what extent do the amounts of litterfall and pruning residues in AFS exceed those of MCS, and how much increase in N supply is achieved by including N-fixing trees in AFS?

2.3. Material and methods

2.3.1. Study site and trial description

The FiBL long-term trial in 'Sara Ana' (15°27'S, 67°28'W) was started in October 2007. It is located in the lowlands of the Alto Beni region in the north-eastern foothills of the Bolivian Andes. The valley of the river Alto Beni was state colonised in the 1960s and is now the main cacao production area in Bolivia. The cacao trees of the long-term experiment were planted in October 2008 and the following cacao production systems were compared: (i) MCS (under full sun) with conv management (with synthetic fertiliser, pesticides, herbicides, manual weeding) = MCS conv, (ii) MCS (under full sun) with org management (compost, legumes, cover crops, bio-control, manual weeding) = MCS org, (iii) AFS (including diverse trees) with conv management (synthetic fertiliser (50% compared to MCS), leguminous trees, cover crops and shade trees, pesticides, herbicides) = AFS conv and (iv) AFS (including diverse trees) with org management (compost (50% compared to MCS), leguminous trees, cover crops and shade trees, bio-control, manual weeding) = AFS org (Schneider et al., 2017).

The valley bottom of the Alto Beni river is at 350 m to 490 m a.s.l. and it is surrounded by the mountain chains of the eastern Andes. The climate is humid tropical with a dry

season from April to September and two arid months (June and July). The mean annual precipitation is 1540 mm, and the average monthly air temperature ranges between 22° C in July and 27° C in December (Elbers, 2002). The natural vegetation of the Alto Beni region is composed of nearly evergreen rainforest (Seidel and Vargas, 1994). The research site is at approximately 380 m a.s.l., situated on a flat subrecent river-terrace of the Alto Beni river. The soils are Luvisols and Lixisols with loamy to clayey–loamy texture, having clay contents of 17 to 35%. Below 50 cm depth, the soils are strongly clayey and have bulk densities up to 1.9 g cm⁻². The argic horizons often show a stagnic colour pattern and are saturated with water during the rainy season. Soil pH (in water) ranges from 5 to 8. At the time of establishment of the trial in 2008, the average SOC content (1.5%) and soil N content (0.16%) in the upper 25 cm of the soils did not differ between the production systems. From 2008 to 2014, both SOC and soil N contents increased, and differences between org (1.7% C; 0.19% N) and conv (1.5% C; 0.16% N) management were detected.

The long-term experiment is arranged in a randomised complete block design with four replicates (Schneider et al., 2017). The factors are ‘crop diversity’ (MCS vs. AFS) and ‘management practice’ (conv vs. org). Gross trial plots are 48 × 48 m, corresponding to 2304 m², while net plots are 24 × 24 m, corresponding to 576 m² (Figure 4). Thirty-six cacao trees were planted per net plot, using 12 different national and international clones (grafted) and hybrids (seeded), spaced at 4 × 4 m (625 trees ha⁻¹), which is a common spacing in the study area (Schneider et al., 2017). After the cacao was planted, perennial soybean (*Neonotonia wightii* (Wight and Arn.) J.A. Lackey) was sown as a ground cover crop for weed control in the organically managed systems. Weed control in the conventionally managed systems was done by herbicide application. Cooking bananas (*Musa × paradisiaca* (L.)) were planted to ensure initial shading of young cacao plants in both AFS and MCS at the same spacing of 4 × 4 m. The cooking bananas were removed at the end of 2011. They were replaced by dessert bananas but only in the AFS (Schneider et al., 2017). In addition, 13 AFS trees were planted in each AFS net plot, including fruit and timber trees and palms (i.e., *Erythrina* spp. (L.), *Euterpe precatoria* (Mart.), *Garcinia gardneriana* ((Planch. and Triana) Zappi), *Inga* spp. (Mill.), *Myroxylon balsamum* ((L.) Harms), *Nephelium lappaceum* (L.), *Persea americana* (Mill.), *Theobroma*

grandiflorum ((Willd. ex Spreng.) K. Schum.)). These trees were planted in between the cacao trees in the first year in a uniform pattern with a distance of 8 m between the cacao trees, corresponding to 225 trees ha⁻¹ (Figure 4). *Erythrina*, a fast-growing leguminous tree was the predominant tree species in the net plots, making up 6 out of 13 trees.

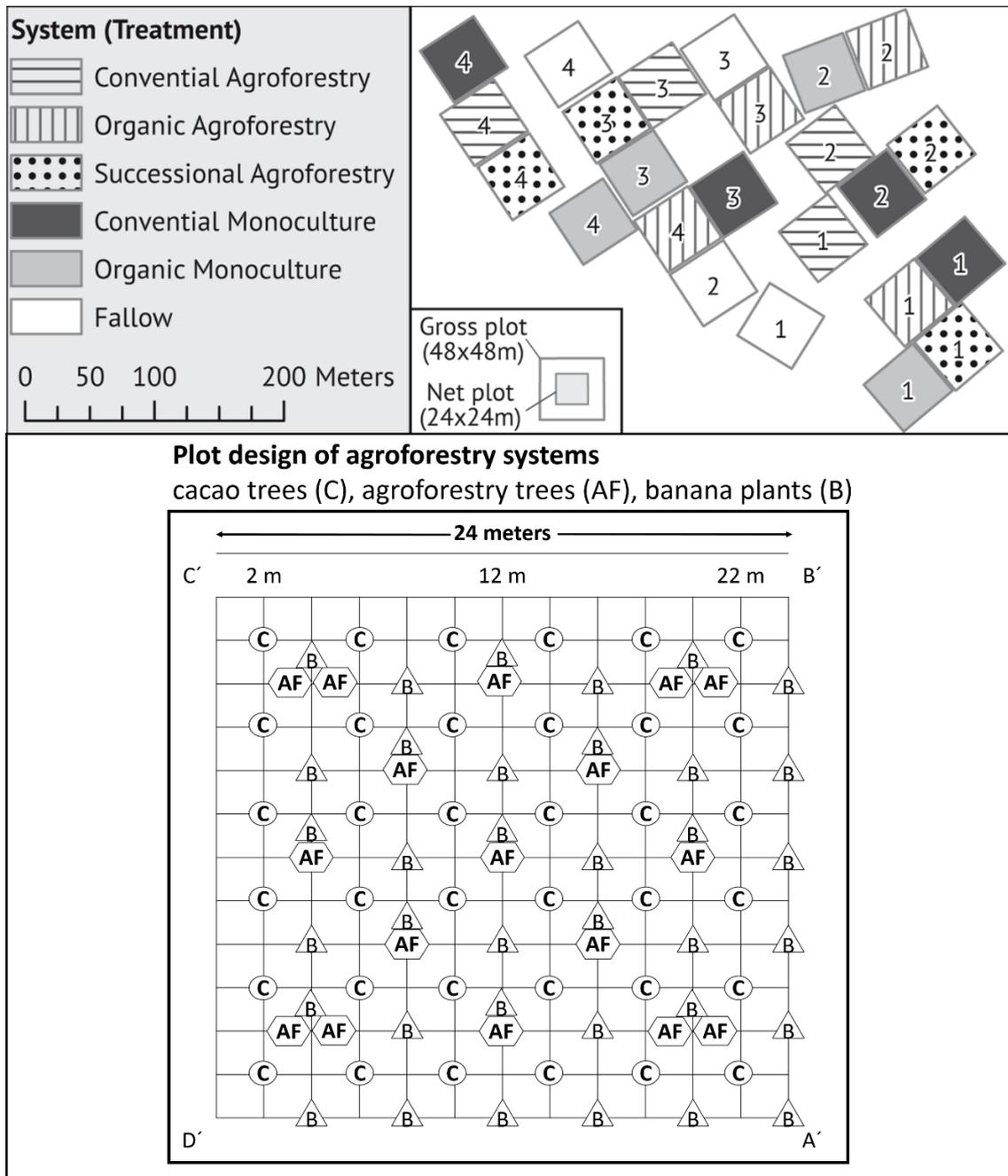


Figure 4: Experimental setup of the FiBL long-term field experiment in Alto Beni, Bolivia. Comparing cacao monoculture systems and agroforestry systems under conventional versus organic management as well as plot design of the agroforestry systems. Numbers in plots represent block (replication), minimum distance between two plots = 2 m, letters represent location of cacao trees (C), agroforestry trees (AF) and banana plants (B) (modified after Schneider et al., 2017).

The cacao trees and bananas were pruned two to three times per year between February and August. The AFS tree pruning was performed once a year at the end of the rainy season (August/September) (Schneider et al., 2017). The pruning residues of the cacao, banana and AFS trees were roughly chopped and left in the plots in all production systems. In the MCS conv systems, the cacao trees were fertilised with 150 kg ha⁻¹ year⁻¹ Blaukorn (BASF, Germany, 12–8–16–3 N–P2O5–K2O–MgO). In the MCS org systems, locally prepared compost was applied (8 Mg ha⁻¹, 24–17–20–18 kg ha⁻¹ N–P–K–Mg). The AFS received half of the amount of fertiliser used in the MCS. Mineral fertiliser was applied twice a year at the beginning and end of the rainy season, while compost was applied once a year at the onset of the rainy season (Schneider et al., 2017).

2.3.2. Aboveground biomass (AGB) and root biomass (RB)

The AGB was estimated in 2011 and in 2015, before pruning was carried out at the end of the dry season. The inventory method mainly followed the recommendations of Pearson et al. (2005). The AGB cacao trees, AFS trees and palms, bananas, herbal and shrub layer, litter layer and deadwood were quantified in the net plots. The AGB (kg tree⁻¹) of each single tree was estimated non-destructively by allometric equations according to Andrade et al. (2008). Cacao tree diameter (d , in cm) was measured at a height of 30 cm, and AGB was estimated from the allometric equation developed by Andrade et al. (2008) (Eq. 1). For plants with stems that ramified below 30 cm, the diameters of all ramifications were measured, and a generalised stem diameter was calculated using Eq. (2) after MacDicken et al. (1991). In the AFS, the tree diameters at breast height (dbh, 130 cm) and the heights (h , in m) of all AFS trees and palms were measured. The AGB of *Inga* spp. was calculated using Eq. (3), whereas Eq. (4) was applied to all other AFS trees (Segura et al., 2006). The AGB of the palm *Euterpe precatorea* (Mart.) was calculated by the use of Eq. (5) for asai palms, after Pearson et al. (2005). The biomass of bananas was estimated using Eq. (6) after van Noordwijk et al. (2002). The AGB estimates of the single trees per net plot were summed and the AGB in (Mg ha⁻¹) was calculated for each component (i.e., cacao trees, AFS trees, bananas and palms).

$$AGB = 10^{(-1.625+2.63*\log(d30cm))} \quad (R^2 = 0.98) \quad (1)$$

$$D = (d1^2 + d2^2 + \dots + dn^2)^{0.5} \quad (2)$$

$$\text{Log10 biomass} = -0.889 + 2.317 (\log 10 \text{ dbh}) \quad (R^2 = 0.96) \quad (3)$$

$$\text{Log10 biomass} = -0.834 + 2.223 (\log 10 \text{ dbh}) \quad (R^2 = 0.93) \quad (4)$$

$$AGB = 6.666 + 12.826 \times h^{0.5} \times \ln(h) \quad (5)$$

$$AGB = 0.03 \times \text{dbh}^{2.13} \quad (6)$$

In addition, within each net plot, destructive sampling of the herb, shrub and litter layers was carried out at four points along a diagonal transect (33.9 m) through the net plot. Litter (dead plant material above the mineral soil with a diameter <10 cm), and herbs and other living plants with a stem diameter <10 cm were collected separately inside a 50 × 50 cm square frame. All collected plant material was weighed, oven dried at 70° C until constant weight and weighed again for the dry weight. The data from the four points along this transect were averaged. Additionally, all deadwood with a diameter >10 cm that occurred along the transect was measured (Pearson et al., 2005).

The root biomass (RB) of the cacao trees was indirectly estimated based on the AGB. Norgrove and Hauser (2013) reported that AGB of cacao trees contributed 87% to the total plant biomass. The contribution of roots to the total plant biomass included 5% from the taproot and 8% from other roots. These proportions were obtained using a biomass partitioning model for cacao developed by Zuidema et al. (2005). The RB of the AFS trees was estimated using Eq. (7) after Cairns et al. (1997).

$$RB = \exp(-1.0587 + 0.8836 \ln(AGB)) \quad (7)$$

AGB and RB were converted into AGC and root carbon (RC) stocks through multiplication by 0.5 (Pearson et al., 2005). The same factor was used for converting the AGB of the herbs, shrubs and the litter layer into C stocks.

2.3.3. Litterfall and pruning residues

The amounts of litterfall and pruning residues differ between cacao varieties (Daymond et al., 2011) and AFS tree species. As a complete survey of all trees was not feasible within this study, one cacao variety, the Bolivian clone Ila-22, was selected. This local cultivar performs well in both MCS and AFS (Schneider et al., 2017). In addition, the

amounts of litterfall and pruning residues were quantified for *Erythrina*, as it was the predominant AFS tree species in the net plots, even though other AFS trees were also pruned. *Erythrina* trees can be significantly pruned and will still regrow very quickly over the rainy season. The cacao trees in the AFS were randomly selected, whereby only cacao trees within the area of influence of *Erythrina* trees were included. Circular litter traps with an area of 0.25 m² were installed at a distance of 0.5 m from stems and 0.5 m above the soil surface. In the MCS, the traps were placed under three cacao trees per net plot, while in the AFS, two traps were placed under cacao trees and two under *Erythrina* trees. Litter was collected monthly from October 2014 until September 2015. The litter of each trap was dried separately for 72h at 70° C and weighed. Annual litterfall per hectare was calculated based on the degree of canopy coverage in the different production systems. Canopy coverage was calculated by analysing 24 hemispherical photographs per plot, which were taken before (July) and after the main pruning of the cacao and AFS trees (October) in 2014 and 2015. Photographs were taken at 1.3 m aboveground along a V-shaped transect within each net plot. Data were computed for three degrees of coverage occurring over the course of a year, i.e., maximum coverage (before pruning), minimum coverage (after pruning) and an intermediate stage of coverage. Litterfall in the AFS was not separated into cacao and AFS tree litter. Litter biomass was converted into amounts of C through multiplication by a factor of 0.5.

The pruning residues of cacao and AFS trees were recorded separately during the main pruning event before the start of the rainy season. For this purpose, the pruning residues of two randomly selected cacao trees (clone Ila-22) per net plot were collected. In addition, the pruning residues of two *Erythrina* trees were collected in each AFS net plot. The pruning residues were divided into leaves and branches, the fresh biomass of which was determined. A subsample of each fraction was dried for 72 h at 70° C to estimate the dry mass of the pruning residues. The amount of AFS tree pruning residues per hectare was calculated based on 13 trees per net plot, using the amounts of pruning residues per tree obtained for *Erythrina*. This approach includes a simplification of the production system, as only 6 out of 13 AFS trees were *Erythrina* trees. The C and N contents of the pruning residues were analysed by use of a CHN analyser (PerkinElmer). The amounts of pruning residues were converted into amounts of C by multiplying their

biomass by their measured C content.

2.3.4. Data analysis

All statistical analyses were performed by the use of R (3.2.3) and RStudio (0.99.486) software (R Core Team 2015) using the package 'lme4' (Bates et al., 2015) and 'lmerTest' (Kuznetsova et al., 2015). All datasets were tested for a normal distribution using the Shapiro–Wilk test. In case of a non-normal distribution, the skew of the respective dataset was calculated, and the dataset was transformed as recommended by Webster (2001). Linear mixed-effects models were applied for AGC stocks, litterfall and pruning. Crop diversity and management practices entered the model as fixed factors, whereas the repetition blocks were considered as random factors. Differences between the years were determined by including the factor year as a fixed factor in the linear mixed-effects models. An ANOVA was carried out for each model. Datasets showing differences between management practices or crop diversity were subjected to pairwise comparisons of least square means (LSMeans) using the 'lsmeans' function of the 'lsmeans' package (Lenth and Hervé, 2015). The significance level was set to $\alpha = 0.05$. The R package 'ggplot2' was used for producing graphs (Wickham, 2009).

2.4. Results

2.4.1. Aboveground carbon (AGC) and root carbon (RC) stocks

In 2011, 3 years after the cacao trees were planted, the AGC in the MCS ranged from 6.7 to 8.3 Mg C ha⁻¹, while the total AGC (of all plants) in the AFS was more than 11 Mg C ha⁻¹ irrespective of the management style (Table 1). However, AGC of cacao trees in the MCS exceeded that of cacao trees in the AFS, where the AGC stocks of cacao trees contributed only 5–12% to the total AGC. Another four years later, in 2015, the amounts of total AGC in the AFS were generally greater than in 2011 ($P < 0.001$). The overall trends showed relative differences between the management variants similar to those in 2011, which had greater amounts of AGC in the AFS (26 Mg C ha⁻¹) than in the MCS (7–8 Mg C ha⁻¹) (Table 1). The AGC stocks of the AFS trees showed a six-fold increase from 2011 to 2015 both under conv and org management ($P < 0.001$). The AGC contribution of AFS trees to total AGC in 2015 was 11 Mg C ha⁻¹ (43%) in the org and 12 Mg C ha⁻¹ (47%) in the conv AFS regimes (Figure 5). In MCS, cacao trees in 2015 contributed 85% to the total AGC under conv management and 70% to total AGC under org management. In the AFS, cacao trees contributed only 14% of the total AGC. The C stocks of cacao trees in MCS (~6Mg C ha⁻¹) exceeded those of cacao trees in AFS (3–4 Mg C ha⁻¹). The total AGC in MCS did not change significantly between 2011 and 2015, while total AGC in AFS increased. No significant differences between org and conv management with respect to total AGC were observed. In 2011, the cacao tree RC ranged between 0.13 Mg C ha⁻¹ (in conv MCS) and 0.10 Mg C ha⁻¹ (in org AFS), not yet showing a significant difference. However, between 2011 and 2015, the amounts of cacao tree RC increased up to eight times in MCS and up to six times in AFS. The RC of all trees in the AFS increased likewise (Table 1).

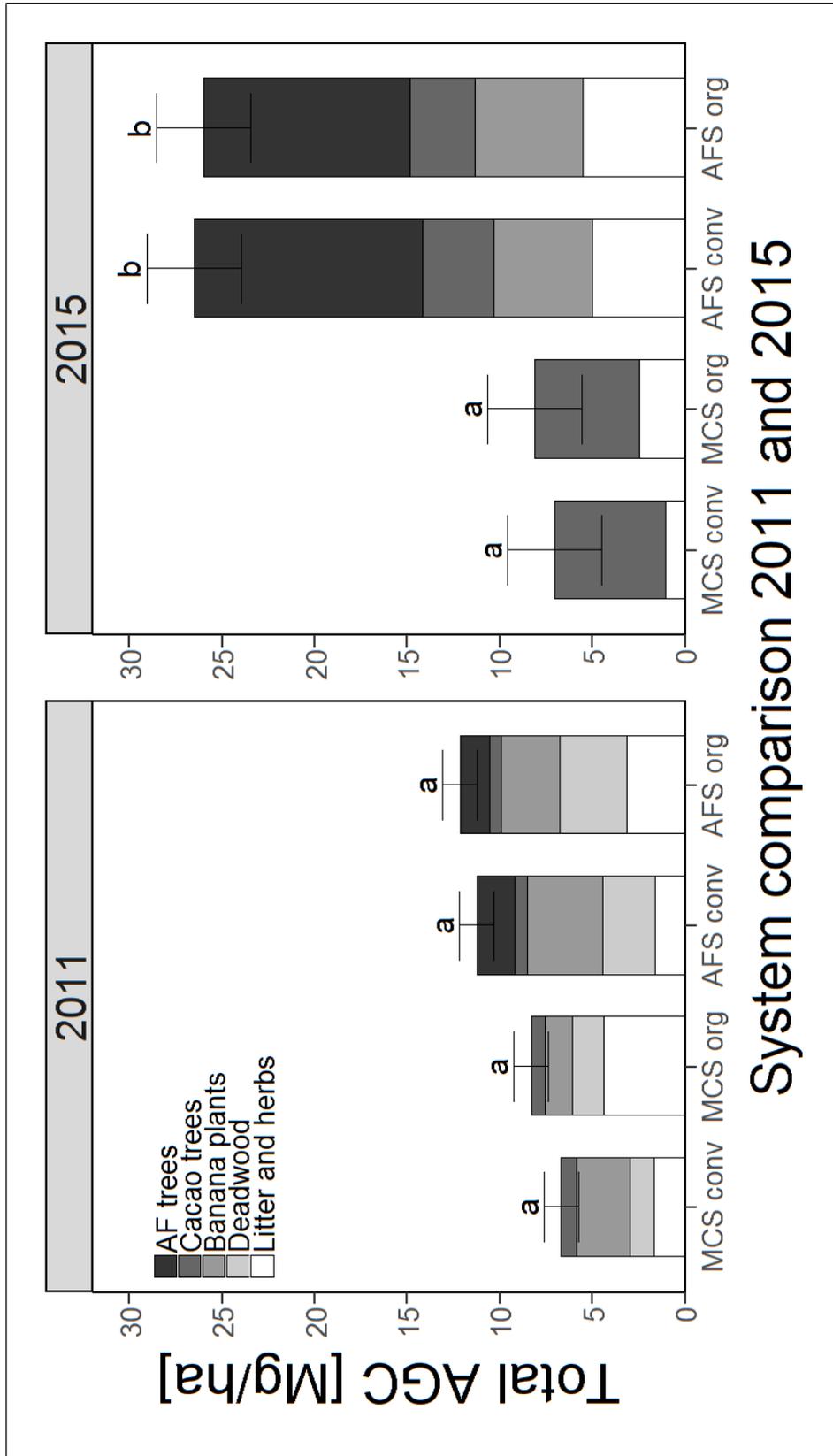


Figure 5: Aboveground carbon (AGC) of the four different cacao production systems for 2011 and 2015. Cumulative bars represent the mean total AGC for the different summed carbon stocks (litter and herbs, deadwood, banana plants, cacao trees, AFS trees). MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management. Means and standard errors presented are non-transformed data. Lower case latin letters indicate significant differences ($P \leq 0.05$) between the production systems.

2.4.2. Annual litterfall

The temporal pattern of litterfall over the year was similar in all production systems. The monthly maximum litterfall was at the end of the dry season in August, while the smallest amounts of litter were recorded during the rainy season in December and January. The total annual litterfall of the AFS significantly exceeded that of the MCS ($P = 0.01$) (Figure 6a). Conventional and org management had no effect on the total annual litterfall ($P = 0.2$). However, the recorded litterfall did not include chopped branches, twigs and husks produced during routine cacao tree phytosanitary treatment, as these were distributed around the trees and not caught in the litter traps. Thus, these components were recorded as constituents of the litter layer but not considered in the annual litterfall.

2.4.3. Pruning residues

The C in the pruning residues of the AFS contained 3.5 Mg C ha^{-1} under org management and 4.3 Mg C ha^{-1} under conv management. Pruning residues in the MCS comprised only 2.6 Mg C ha^{-1} under org management and 3.3 Mg C ha^{-1} under conv management (Figure 6b; Table 1). The increased C stocks in pruning residues of AFS primarily resulted from the abundant pruning residues of the *Erythrina* trees (~60%). The C stocks of the pruning residues of cacao plants were slightly lower in the AFS than in the MCS (Table 1).

Cacao leaves showed higher N contents in AFS than in MCS ($P = 0.01$). The significant interaction between crop diversity and management showed that there were no differences in the N content between AFS and MCS under org management, but N content was higher in the AFS compared with the MCS when conventionally managed (Table 1). N contents in cacao branches were higher under org than under conv management ($P < 0.02$), and cacao branches in AFS had higher N contents than cacao branches in MCS ($P < 0.04$). Amounts of N in cacao pruning residues were $0.06 \text{ Mg N ha}^{-1}$ in the AFS and approximately $0.10 \text{ Mg N ha}^{-1}$ in the MCS, but this difference was not significant ($P = 0.11$). The amounts of N in pruning residues of *Erythrina* trees (~ $0.06 \text{ Mg N ha}^{-1}$) did not differ between conv and org management (Table 1). Similarly, the amount of N in the total AFS pruning residues did not differ between conv and org management (~ 0.1 Mg N ha^{-1} , Figure 6c).

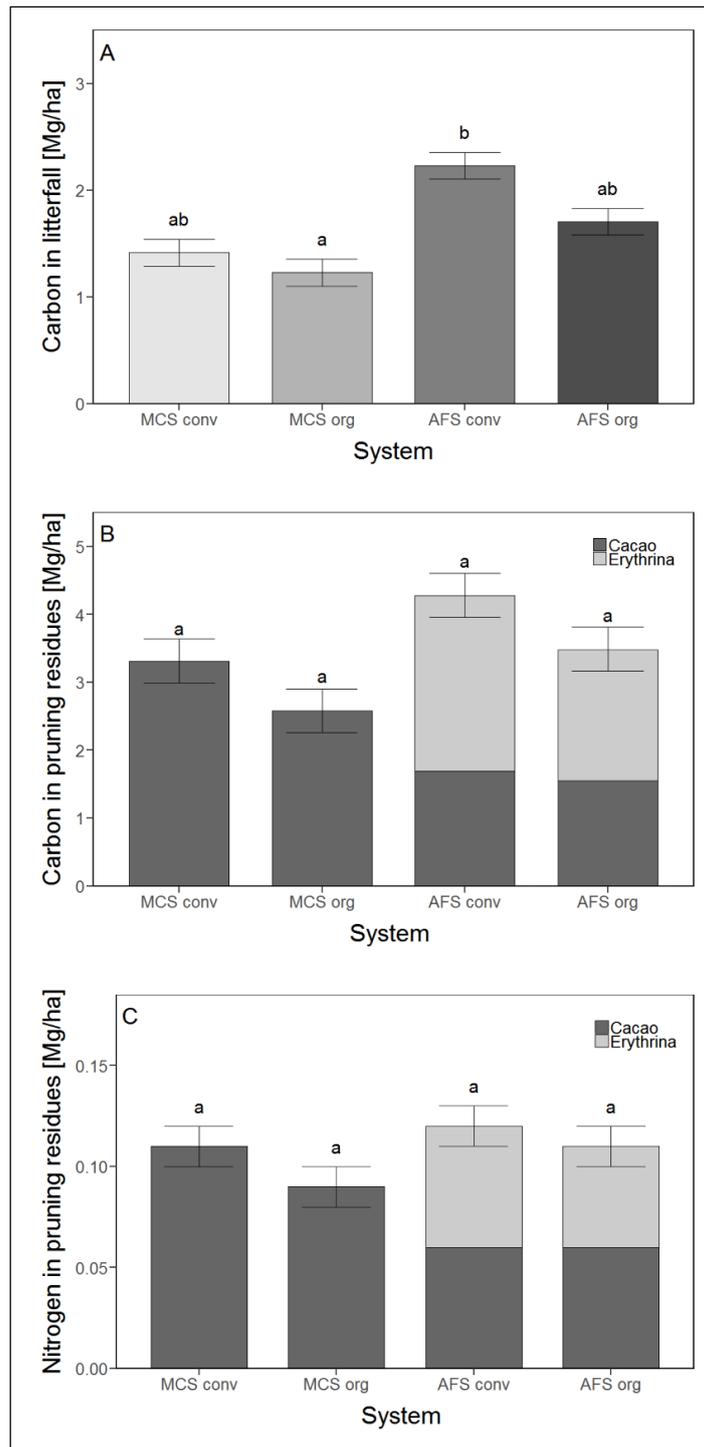


Figure 6: (a) Carbon contents in total annual litterfall, (b) carbon and (c) nitrogen contents in pruning residues of four different cacao production systems.

Bars show the summed pruning residues of cacao and *Erythrina* trees. MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management. Means and standard errors presented are non-transformed data. Lower case latin letters indicate significant differences ($P \leq 0.05$) between the production systems.

Table 1: Results of the linear mixed models and mean \pm SE for aboveground (AGC) and root (RC) carbon stocks (years 2011 and 2015), Carbon and Nitrogen in pruning residues (2015), and litterfall for cacao and erythrina trees (2015) for four different cacao production systems.

	F value											
	Crop diversity (Crop)	Management practice (Man)	Crop \times Man	MCS conv	Dif.	MCS org	Dif.	AFS conv	Dif.	AFS org	Dif.	
C stocks 2011 (Mg C ha ⁻¹)	Litter and herbs	2.75	30.40 ***	2.42	1.67 \pm 0.53	a α	4.39 \pm 0.47	b α	1.63 \pm 0.47	a α	3.16 \pm 0.41	ab α
	Deadwood	3.31	<0.01	0.083	1.28 \pm 0.52	a	1.72 \pm 1.52	a	2.79 \pm 0.68	a	3.60 \pm 1.89	a
	Cacao trees	9.63 *	0.97	0.97	0.85 \pm 0.17	a α	0.75 \pm 0.10	a α	0.65 \pm 0.11	a α	0.65 \pm 0.09	a α
	AFS trees	-	9.31 *	-	-	-	-	-	2.07 \pm 0.09	a α	1.58 \pm 0.13	b α
	Banana plants	3.93	2.59	0.13	2.90 \pm 0.51	a	1.45 \pm 0.23	a	4.10 \pm 1.20	a α	3.17 \pm 0.65	a α
	Total AGC	6.50 *	0.59	0.04	6.70 \pm 0.79	a α	8.31 \pm 1.49	a α	11.24 \pm 2.07	a α	12.17 \pm 1.94	a α
C stocks 2015 (Mg C ha ⁻¹)	Total RC	575.58 ***	10.85 **	6.89 *	0.13 \pm 0.02	a	0.11 \pm 0.02	a α	0.71 \pm 0.01	b α	0.58 \pm 0.04	c α
	Litter and herbs	26.71 ***	3.23	4.35	1.04 \pm 0.21	a α	2.45 \pm 0.43	ab β	4.99 \pm 0.49	b β	5.54 \pm 0.85	b α
	Deadwood	-	-	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Cacao trees	68.43 **	1.82	0.93	6.00 \pm 0.42	a β	5.68 \pm 0.51	a β	3.85 \pm 0.65	b β	3.48 \pm 0.52	b β
	AFS trees	-	0.72	-	-	-	-	-	12.33 \pm 0.67	a β	11.18 \pm 1.18	a β
	Banana plants	-	0.44	-	0.00	0.00	0.00	0.00	5.35 \pm 0.42	a α	5.81 \pm 0.55	a β
Cacao trees	Total AGC	259.08 ***	0.56	1.48	7.04 \pm 0.55	a α	8.12 \pm 0.13	a α	26.53 \pm 1.57	b β	26.01 \pm 3.29	b β
	Total RC	355.36 ***	1.78	0.92	0.90 \pm 0.06	a	0.85 \pm 0.08	a β	3.52 \pm 0.16	b β	3.22 \pm 0.19	b β
	LeAFSN [%]	9.85 *	3.39	10.19 *	2.01 \pm 0.06	a	2.32 \pm 0.05	b	2.40 \pm 0.09	b	2.31 \pm <0.01	ab
	Branch N [%]	6.29 *	8.32 *	0.01	0.95 \pm 0.12	a	1.17 \pm 0.05	ab	1.14 \pm 0.09	ab	1.38 \pm 0.02	b
	Pruning N (t ha ⁻¹)	2.54	0.12	0.24	0.11 \pm 0.03	a	0.09 \pm 0.02	a	0.06 \pm 0.01	a	0.06 \pm 0.03	a
	Pruning C (t ha ⁻¹)	4.43	0.81	0.19	3.31 \pm 0.72	a	2.58 \pm 0.69	a	1.69 \pm 0.39	a	1.55 \pm 0.67	a
Erythrina trees	LeAFSN [%]	-	<0.01	-	-	-	-	-	2.77 \pm 0.13	a	2.87 \pm 0.21	a
	Branch N [%]	-	4.14	-	-	-	-	-	1.13 \pm 0.06	a	1.25 \pm 0.05	a
	Pruning N (t ha ⁻¹ y ⁻¹)	-	0.45	-	-	-	-	-	0.06 \pm <0.01	a	0.05 \pm 0.01	a
	Pruning C (t ha ⁻¹ y ⁻¹)	-	0.94	-	-	-	-	-	2.59 \pm 0.20	a	1.93 \pm 0.65	a
	Pruning N (t ha ⁻¹ y ⁻¹)	0.81	0.37	0.07	0.11 \pm 0.03	a	0.09 \pm 0.02	a	0.12 \pm 0.01	a	0.11 \pm 0.03	a
	Pruning C (t ha ⁻¹ y ⁻¹)	2.99	1.91	<0.01	3.31 \pm 0.72	a	2.58 \pm 0.69	a	4.28 \pm 0.19	a	3.49 \pm 0.73	a
Litterfall C (t ha ⁻¹ y ⁻¹)	10.90 **	2.04	0.17	1.42 \pm 0.17	ab	1.23 \pm 0.16	a	2.23 \pm 0.12	b	1.71 \pm 0.24	ab	

MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management. Dif. = Different lower case Latin letters within lines indicate significant differences ($p \leq 0.05$) between the production systems; lower case Greek letters, within columns, indicate significant differences ($p \leq 0.05$) between the year 2011 and 2015 within a production system; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

2.5. Discussion

2.5.1. Aboveground carbon (AGC) in cacao monoculture systems (MCS) and agroforestry systems (AFS)

Seven years after establishing the FiBL long-term experiment, the cacao AFS comprised significantly more AGC (21 Mg C ha⁻¹) than the cacao MCS (Table 1; Figure 5). This difference is mainly due to the amounts of C stored in the AFS trees. However, the AGC of the AFS was still only one-third of the tree-AGC of trees in the surrounding natural forests (~ 65 Mg C ha⁻¹; Yaffar, 2014). Nevertheless, it had only been 7 years since the establishment of the experimental plots, and a further increase in AGC can be expected, as total amounts of AGC up to 50 Mg C ha⁻¹ have been reported from fully developed AFS in the same region (Jacobi et al., 2014).

The contribution of the AFS trees to the total AGC was comparable to that reported from 10-year-old AFS with *Erythrina poeppigiana* (contributing 18.95 Mg C ha⁻¹, corresponding to 43% of total AGC) in Costa Rica (Beer et al., 1990). In contrast, much greater contributions of AFS trees to AGC have been reported for trees in multi-shade cacao systems in Indonesia (39 Mg C ha⁻¹, 81% of total AGC) (Abou Rajab et al., 2016), and even greater contributions of AFS trees to AGC were observed in mature shaded cacao systems in Cameroon (contributing >90 Mg C ha⁻¹, corresponding to ~90% of total AGC) (Norgrove and Hauser, 2013; Saj et al., 2017).

In our study in Bolivia, the C stocks of cacao tree biomass increased more rapidly in MCS than in AFS (Figure 5). Three years after the experimental plots were established, the biomass of cacao trees in MCS exceeded that of cacao trees in AFS by up to 25%. After 7 years, the AGC stocks of cacao trees in MCS exceeded those of cacao trees in AFS 1.5 times. Thus, the difference in biomass between the cacao trees in MCS and AFS considerably increased from 2011 to 2015. Enhanced increases in cacao tree biomass under full-sun conditions have also been reported by Tschardt et al. (2011). Comparatively low growth rates of the cacao trees in the Bolivian trial may explain the relatively lower cacao yield found in the AFS compared to the MCS (Armengot et al., 2016). However, Abou Rajab et al. (2016) showed that cacao yields do not necessarily decrease under shading. In our study, the higher tree density and shade level of approximately 50% (Schneider et al., 2017), in the AFS compared to the MCS, may

explain the slower growth rates in biomass of the cacao trees. In detail, the MCS comprised 625 cacao trees ha⁻¹, while the AFS comprised a total of 850 trees per hectare, plus an additional 625 banana plants ha⁻¹. This setting might have reduced cacao tree growth due to limited insolation and interspecies competition for nutrients. The importance of light intensity for cacao tree growth has been emphasised by Wood and Lass (1985). While moderate shade is important for sensitive young cacao trees, cacao tree biomass production rates show a general increase with light intensity of up to 25% (Abou Rajab et al., 2016; Mortimer et al., 2017; Triadiati et al., 2007). Since it is known that cacao trees have a low light saturation level of approximately 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Daymond et al., 2011), shading between 40 and 70% is considered optimal (Beer et al., 1998). Increased biomass of 8-year-old cacao trees under moderate shade (30 AFS trees ha⁻¹) relative to that of trees in MCS was reported by Isaac et al. (2007). To provide sufficient insolation for optimal cacao tree growth, it is therefore advisable to establish a pruning management regime that is adapted to local climatic conditions as well as to the flowering and growth phases of the cacao trees. AFS comprise diverse kinds of trees, including easy-to-prune and quickly growing trees, enabling the most flexible adaptability to a wide range of situations (Schroth et al., 2001; Tschardtke et al., 2011). In addition, when selecting AFS trees, compatibility with respect to nutrient competition must be ensured, which may otherwise limit the growth and productivity of cacao trees. The overall effect of various AFS trees on cacao trees also depends on the general availability of water and soil nutrients, and on competition for light (Beer et al., 1998; Mortimer et al., 2017; Saj et al., 2017; Tschardtke et al., 2011). Furthermore, various studies have shown that AFS trees enhance the overall nutrient cycling in AFS (Isaac et al., 2007; Saj et al., 2017; Tschardtke et al., 2011).

In comparable studies, the AGC stocks of cacao trees in AFS ranged from 4 Mg C ha⁻¹ to almost 20 Mg C ha⁻¹ (Abou Rajab et al., 2016; Beer et al., 1990; Isaac et al., 2007; Saj et al., 2017). This wide range of reported AGC stocks is due to differences in tree composition, age, stem density and cacao variety. Moreover, the studies were carried out in various regions with different conditions, in terms of climate, soil properties and water and nutrient supply, making direct comparisons difficult. For instance, in Indonesia, the AGC of 14- to 22-year-old cacao MCS with a total tree density of

approximately 892 trees ha⁻¹ was 7.7 Mg C ha⁻¹ (Abou Rajab et al., 2016), whereas the AGC in 8-year-old cacao MCS in Ghana was 11.4 Mg C ha⁻¹ (Isaac et al., 2007). This amount of AGC is almost twice as large as that of the cacao MCS in the current FiBL experiment in Bolivia, which can be explained by different cacao tree densities (1100 trees ha⁻¹ in the study of Isaac et al. (2007) versus 625 trees ha⁻¹ in the FiBL trial in Bolivia). Considering these differences in tree densities results in similar amounts of AGC stored per cacao tree.

2.5.2. C and N fluxes through litterfall and pruning residues in cacao monoculture systems (MCS) and agroforestry systems (AFS)

The amount of C in the total litterfall of the cacao AFS (org 1.7 Mg C ha⁻¹ and conv 2.2 Mg C ha⁻¹) significantly exceeded those of the cacao MCS (org 1.2 Mg C ha⁻¹ and conv 1.4 Mg C ha⁻¹) (Table 1; Figure 6a). This remarkable difference can be explained by the higher total tree density (sum of cacao trees and AFS trees). The amount of C in the litter was within the lower range of data reported in the literature for plantations of a similar age (Abou Rajab et al., 2016; Beer et al., 1990; Dawoe et al., 2010). The amount of C in litter reported from other studies ranges from 2.4 Mg C ha⁻¹ in Indonesia to 5.2 Mg C ha⁻¹ in 30-year-old AFS in Ghana. Litterfall in AFS in Ghana increased with stand age and reached natural forest levels at an AFS age of 15 years (Dawoe et al., 2010). C in the litter of a natural forest in our study region in Bolivia, over a nine-month period (April to December), amounted to almost 5.5 Mg C ha⁻¹ (Yaffar, 2014), which is more than twice the amount of C in the litter of the AFS in the trial.

In this study, the amounts of C in *Erythrina* pruning residues (2.6 to 4.3 Mg C ha⁻¹) were twice as high as those found in the litter (Figure 6b). The pruning residues in this experiment may have been overestimated, since they were calculated based only on *Erythrina* trees that were pruned much more intensively than other timber and fruit trees that were included in the AFS. Nevertheless, our results match well with data reported for AFS with *E. poeppigiana* in Costa Rica (Beer et al., 1990). The pruning results of our study thus apply to simple AFS, including those with fast-growing legume trees. However, various cacao AFS that are not pruned exist around the world (Nair et al., 2017). AFS with a more diverse AFS tree composition, in which only some of the tree species are pruned, likely have a greater permanent tree biomass than systems with

heavily pruned AFS trees, while C and N fluxes are particularly enhanced in strongly pruned AFS. Therefore, fast-growing, easy-to-prune AFS trees may also be used to improve the nutrient supply for young AFS that are installed on poor soils and cleared forest areas. However, until now little attention has been paid to the role of pruning residues from different AFS trees for C and nutrient fluxes in cacao plantations. In some studies, the pruning residues were either included as part of the litterfall or assumed to be negligible (Abou Rajab et al., 2016) partly because cacao plantations are not always systematically established with shade trees, and if so, this does not necessarily include a purposeful introduction of certain tree species. Many different types of local and indigenous AFS exist, including cultivation of cacao trees under remaining natural forest trees or tree species that cannot be pruned strongly enough to provide optimal light conditions for the cacao trees (Nair et al., 2017).

The relative proportion of the pruning residues of cacao tree biomass was similar in all systems (45–55%). The greater biomass of the cacao trees in the MCS corresponded to a higher proportion of cacao tree pruning residues compared AFS's tree pruning residues; however, the lower amounts of cacao tree pruning residues in the AFS were compensated by the other AFS trees.

Pruning is important in AFS, for ensuring that sufficient sunlight reaches the cacao trees, for pest and disease control and for the prevention of soil erosion by a protective cover of pruning residues (Johns, 1999; Norgrove and Hauser, 2013; Zuidema et al., 2005). Data in the literature indicate that the C in pruning residues and litterfall from leguminous AFS trees varies from 1.7 to 7 Mg C ha⁻¹ a⁻¹. The associated amounts of N range from 60 to 340 kg ha⁻¹ a⁻¹ (Beer, 1988). In the FiBL trial in Bolivia, the tree-soil N fluxes via litterfall and pruning residues amounted to 90–120 kg N ha⁻¹ a⁻¹, both in MCS and AFS (Table 1). The N contents of the pruning residues of cacao clone Ila - 22 (2.0 to 2.4% N) were similar to those reported from a cacao experiment by Kähkölä et al. (2012), in which the cacao trees were inoculated with *Inga edulis* (Mart.). The pruning residues in that experiment contained 2.6% N for inoculated and 2.1% N for non-inoculated cacao trees. Comparably high leaf N contents (2.4 to 3.2% N) were also reported by Daymond et al. (2011) for different cacao clones used in a greenhouse experiment, whereby the N contents depended on the clones. Triadiati et al. (2007) reported lower N contents for

cacao leaves in different cacao AFS with varying canopy coverages (1.2 to 1.5% N). They concluded that increased light intensity leads to enhanced biomass production, and that the presence of N-fixing trees leads to increased soil N contents, from which the cacao trees also benefit. In the FiBL trial, the conventionally managed MCS showed the lowest leaf N contents (2.0% N). All the other production systems in which leguminous plants were included exhibited somewhat higher leaf N contents that were very similar to each other (2.3% for both MCS and AFS under org management, and 2.4% for AFS under conv management) (Table 1). Branches of cacao clone Ila-22, in both AFS and in organically managed MCS, exhibited higher N contents than those in conventionally managed MCS. These results suggest that under the conditions of this study, N-fixing *Erythrina* trees in the AFS, and likewise leguminous cover crops in organically managed systems, lead to increased soil N contents and uptake by the cacao trees. Kähkölä et al. (2012) found that root litter of the leguminous tree *Inga edulis* (Mart.) represents a more important and more quickly available N source for cacao trees than the leaf litter of the same tree. However, the N contribution of leaf litter and root litter from leguminous plants in organically managed crop production systems to increases in observed soil N needs further investigation. The beneficial effect of N-fixing leguminous trees in AFS is particularly relevant if N fertiliser input is low, and soils are poor in N (Saj et al., 2017; Schroth et al., 2001). However, including leguminous plants in crop production systems can also lead to enhanced acidification of the rhizosphere. Moreover, *Rhizobium* requires large amounts of P and may thereby lead to a decrease in P availability for cacao trees (Mortimer et al., 2017; Yan et al., 1996). These decreases in pH and P availability can be mitigated by ensuring a regular return of biomass through pruning (Yan et al., 1996).

In the FiBL long-term field experiment, the total plant–soil N fluxes through pruning residues exceeded the N inputs through N fertilisation up to 10 times. In the cacao MCS, the N returns through pruning residues derived only from the cacao trees, whereas in the AFS, leguminous trees contributed 50% of the plant–soil N flux through pruning residues, hence generating a considerable N gain for the system. Therefore, the system's N cycling is largely influenced by the composition of AFS trees and by the pruning practices (Schroth et al., 2001). The increased N availability in AFS from leguminous trees

in turn leads to an increase in biomass production (Beer, 1988). Moreover, Beer et al. (1990) observed an increase of SOM contents by 21% under pruned *Erythrina* trees over a 10-year period.

2.6. Conclusion

The systematic study of C sequestration and C fluxes in conventionally and organically managed cacao AFS and MCS of the FiBL long-term field experiment in Bolivia confirmed that cacao AFS have a greater potential for C sequestration compared to cacao MCS. The experimental setup allowed for the quantification of these differences and for the identification of their underlying causes, namely, the greater biomass and higher tree density of AFS, especially if fast-growing leguminous trees that can be heavily pruned are included. Pruning of leguminous AFS trees also enhances C and N cycling in the soil–plant system and ensures long-term accumulation of C and N in AFS. This may in turn also lead to an increase in SOM contents over time. In addition, this study showed that leguminous trees and cover crops in organically managed systems can improve the N availability for all AFS plants within a short period of time, as indicated by increased N contents in cacao leaves and branches. Tree density, pruning practices and corresponding sunlight intensity were identified as important factors for the growth of cacao trees. In this study, no considerable advantages were observed for conv over org management with respect to AGB. We concluded that AFS, especially under org management, may be productive systems, with the additional benefits of increased C sequestration and enhanced N supply compared to cacao MCS.

Contributions of authors

Conception and design of the study: Ulf Schneidewind, Felix Heitkamp and Gerhard Gerold. Material preparation, data collection and analysis were performed by Ulf Schneidewind, Felix Heitkamp and Laura Armengot. The first draft of the manuscript was written by Ulf Schneidewind and all authors commented on previous versions of the manuscript.

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3. Organic managed cacao agroforestry systems increase soil carbon and nitrogen levels and microbial biomass within six years after establishment



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Organic managed cacao agroforestry systems increase soil carbon and nitrogen levels and microbial biomass within six years after establishment

3.1. Abstract

Aims

Agroforestry and org farming pursue largely the same goals and methods. Accumulation of biomass for improvement of soil structure and fertility as the basis for agricultural production and avoidance of agrochemicals to preserve biodiversity. Nevertheless, there are still many gaps of knowledge about AFS and org agriculture, especially about the long-term effect.

Methods

In this study, conv and organically managed cacao monocultures (MCS) and agroforestry systems (AFS) are compared in terms of soil organic carbon (SOC) and total nitrogen (N), microbial biomass, and litter decomposition.

Results

SOC and N stocks in the topsoil were significantly higher in org management practices, while there are no differences between the MCS and AFS with the same management practices. A change between the cultivation systems with increasing soil depth was not found. Microbial biomass strongly decreases from the organic surface layer to the subsoil in all systems. Differences in Cmic concentrations became apparent between the four different production systems. In the organic surface layer microbial nitrogen was four times higher in the organically managed systems than in conv systems. The org managed AFS had the highest concentrations of microbial nitrogen. Decomposition rates of cacao and *Erythrina* spp. leaves showed no differences between the systems, but half-life of cacao leaves litter was almost twice of erythrina leaves.

Conclusion

Thus, organically managed cacao AFS lead to healthier soils and should therefore form the basis for production that is not only designed for short-term profit, but also preserves soil ecosystem services in the long-term.

3.2. Introduction

No single definition exists for agroforestry systems (AFS), except that it is an agricultural production system in which trees are combined with other crops or livestock (FAO 2017; ICRAF 2000; Nair 1993). AFS have a long history all over around the world and therefore exist in many different forms and shapes. Depending on the climate region and the products cultivated, AFS can range from a few individual trees per hectare to very diverse systems with a large number of trees. In the tropics, agroforestry involves the integration of trees and other large perennial woody plants into agricultural systems through the maintenance of existing trees, their active planting and the toleration of spontaneous tree regrowth (Schroth et al., 2004). These trees in AFS can perform a variety of ecological functions and also provide a direct economic benefit for the producers (Barrios et al., 2018; Udawatta et al., 2017; van Noordwijk, 2021). Ecological functions include buffering climatic oscillations, protecting soil and water resources, and an internal nutrient cycle (Jose, 2009; Niether et al., 2018; Schneidewind et al., 2019). The direct benefits for the producer are income diversification, food security and food sovereignty (Jacobi 2016; Schneider et al., 2017) Due to these ecological and economic benefits AFS is perceived as a land management system that contributes to United Nations Sustainable Development Goals (van Noordwijk et al., 2018).

Even though the benefits seem obvious, there are widespread prejudices from producers, the industry, and scientists about the profitability of AFS. These prejudices mainly relate to a perceived lower return in AFS in the early years (Schroth et al., 2004). The prejudices against AFS stem from the same arguments that are used to argue against org farming. There is criticism that org farming produces lower yields and therefore requires more land to produce the same amount of food (Connor 2008; Trewavas 2001). Which in turn would lead to higher deforestation rates in the tropics and a loss of biodiversity. In AFS, this may be the case if primary and secondary forests are cleared for the establishment of farms and the focus of the installation is not on open or degraded areas (Martin et al., 2020). However, Seufert and Ramankutty (2017) clearly show the advantages of org agriculture. These advantages range from a positive impact on local biodiversity, to high productivity and livelihoods for poor farmers in certain situations. The mechanisms and the benefits are broadly similar to those of AFS, which

is why this type of agriculture goes hand in hand with many important perennial crops such as cacao and coffee. The SysCom program (<https://systems-comparison.fibl.org/>) has addressed this gap by over a dozen years of successful participatory and production systems research dedicated to the development of sustainable agricultural systems in the tropics (Bhullar et al., 2021).

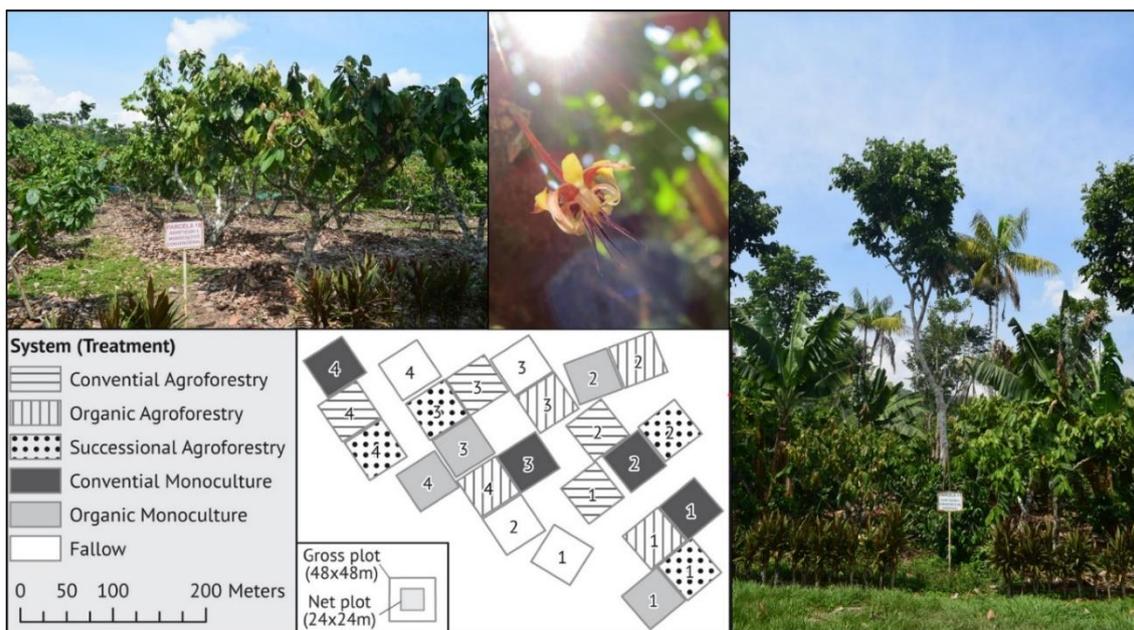


Figure 7: Experimental setup of the FiBL long-term trial (modified after Schneider et al., 2016).

Contrasting conventional cacao monoculture and organic agroforestry system.

However, there are many unanswered questions regarding AFS and org farming, on the positive effects on soil quality in the long term. Soil properties can vary greatly even on a small scale, especially in forest systems (Schöning et al., 2006). Soil quality values can therefore vary greatly from study to study. For instance, Monroe et al. (2016) determined 57 Mg C ha⁻¹ in a 4-year-old cacao and rubber AFS, while Norgrove and Hauser (2013) indicated only 15 Mg C ha⁻¹ in a 35-year-old AFS in Cameroon. The history of the soil thus plays a crucial role in assessing changes in soil C and nitrogen stocks, between different cropping systems. The microbial biomass changes much faster and is a good early indicator (Fließbach and Mäder, 2000; Powlson et al., 1987). Therefore, concentrations can be compared more easily, but also greater differences can be found in arable org managed systems due to long-term management (Fließbach and Mäder, 2000; Lori et al., 2017).

Therefore, it might be useful to shade light on understanding the soil quality changes

between systems over the years in one experimental site. Such it is done in the SysCom long term trail in Bolivia by comparing MCS and AFS in both conv and org farming (Figure 7).

Therefore, with regard to soil quality this article addresses the following questions:

1. Do SOC and N contents differ with respect to crop diversity and management practices?
2. In which cacao production system is microbial biomass highest?
3. Does the incorporation of legume trees, such like *Erythrina* spp., in AFS increase the microbial activity?
4. Is there an effect on litter decomposition due to the cacao production system?

3.3. Materials and methods

3.3.1. Research area and trial description

The field research was conducted at the SysCom Cocoa Research Station in Sara Ana, Alto Beni – Bolivia (15°27' S and 67°28'W). The research station was put into operation in 2008 to study different cacao production systems.

Sara Ana is located in the North-Eastern foothills of the Bolivian Andes. The climate is humid-tropical with a distinctive dry season from mid-April to the end of September (Niether et al., 2018). Annual precipitation is around 1500 mm with mean annual temperature of about 25° C. The temperatures in the different seasons can vary widely with single days with temperatures below 10° C in the dry season and up to 42° C in the rainy season. The natural vegetation is an almost evergreen rainforest with plant species from the Northern moist broadleaf tropical rainforest of the Amazon rainforest. The plant community thus forms the South-Western edge of the Amazon biome.

The Sara Ana research station lies west, adjacent to the Alto Beni river. The flat sub-recent river terrace is located 400 m a.s.l.. Soils are stagnic Luvisols and Lixisols with a loamy to clay-loamy texture and the pH ranges from 5 to 8 (Schneidewind et al., 2019).

The long-term cacao production systems were established at the end of 2008. Different cacao production systems were arranged in a complete randomised block design with four replicates (Schneider et al., 2017).

The difference in cultivation systems ranges from full-sun monocultures (MCS) to agroforestry systems (AFS), both under conventional (conv) and organic (org) management. The plot size is 48 by 48 m, with a net plot of 24 by 24 m, for specific data collection. Cacao trees were planted in a regular grid at a distance of 4 by 4 m in all five production systems (625 trees ha⁻¹). In the AFS various shade, fruit, and timber trees as also palms were planted in between the cacao trees with a spacing of 8 m (227 trees ha⁻¹). Short rotation and fast-growing leguminous trees (*Erythrina* spp. and *Inga* spp.) were planted as well for biomass accumulation and as a nitrogen source, both with a spacing of 8 by 16 m and a planting density of 78 trees ha⁻¹. Bananas (*Musa × paradisiaca* (L.)) were planted in the centre of 4 cacao trees. In org managed systems perennial soybean (*Neonotonia wightii* (Wight and Arn.) J.A. Lackey) was used as ground cover for weed control, biomass accumulation and nitrogen fixation. Mineral fertilizer was applied in the conv systems, while locally produced compost was used in the org systems (Schneider et al., 2017). Cacao trees are pruned three times a year. In January and May, a thinning pruning is made for better ventilation, light incidence, flowering and fruit set. At the end of the harvest season (August/September) a phytosanitary pruning is performed, which prepares the tree for the next harvest period. Pruning residues were chopped and collocated in a distance of 0.5-1 m around the trunk. For agroforestry trees, annual pruning was done at the beginning of the rainy season in December. For more details about the plot design and management practices see Schneider et al. (2017).

3.3.2. Soil sampling and analysis

Soil samples for soil organic carbon (SOC), total nitrogen (N), microbial carbon (C_{mic}) and microbial nitrogen (N_{mic}) analyses were collected at the end of the 2014 rainy season. Soil samples (0–25 cm) were taken under cacao and erythrina trees with a soil auger and separated according to the layer (O-horizon: surface layer of organic matter, A-horizon: topsoil, B-horizon: subsoil). Within each MCS and AFS net plot three cacao trees of a local clone variety Ila-22 was chosen. Additionally, three erythrina trees in the AFS were randomly selected, within the net plot. Around each tree four soil samples were taken in a distance of 0.5 - 1 m from the trunk. For each horizon (O, A and B) and tree type a separate composite sample of about 200g was prepared for each plot. The field moist soil samples were sieved (2 mm) and stored under constant conditions in

Bolivia. Once in Göttingen, the samples were stored at 6° C until the analyses.

A total of 72 samples were analysed (16 cacao samples and 8 erythrina samples per horizon) at the University of Göttingen in the laboratory of the Geographical Institute. Cmic and Nmic was quantified via chloroform-fumigation-extraction (CFE) as described by Vance et al. (1987) and Joergensen (1996). The concentration of C and N in fumigated and non-fumigated soil extracts was measured with a Dima-TOC 100 and Dima-N automatic analyzer (Dimatec, Essen, Germany). Microbial biomass C and N was calculated as follows:

$$\text{Mic} = E/kE$$

where E = (extract from fumigated soil) – (extract from non-fumigated soil) and kE the factor to convert E to Cmic and Nmic. The following conversion factors were used: Cmic = 0,45 (Joergensen 1996) and Nmic = 0,54 (Joergensen and Mueller 1996).

Soil samples for SOC and N were air-dried, ground, and measured by dry combustion using a LECO TruSpec CHNS elemental analyzer (LECO Corporation, St. Joseph, MI, USA). For the calculation of SOC and N stocks, bulk densities for the two layers, A (0-10 cm) and B (10-25 cm), from 2010 were used (unpublished data). For the bulk density, three samples per horizon were collected in 2010 at each of three locations in the border of the 24 by 24 m net plot. The average bulk density for the A and B-horizon were 1.2 ± 0.1 and 1.5 ± 0.1 g/cm².

3.3.3. Leaf litter decay

Leaf litter decomposition was estimated by the litterbag method as described by Bockock and Gilbert (1957) and also by Bärlocher (2005). The experiment was conducted over a period of 12 months and started mid-October 2014 before the rainy season. Fresh cacao and erythrina leaves of the annual tree pruning were collected. Leaves from pruning were selected because annual pruning is an important source of litter input to managed AFS (Schneidewind et al. 2019).

About 10 g of leaves, separated by plot and tree species, were placed into 20 cm x 20 cm nylon mesh bags with 1 mm mesh size.

The litterbags were laid out in a circular pattern under the crown space of the cacao trees (0.5 – 1 m). They were placed on the surface and connected with a string, which

was fixed with a hook in the ground. In the AFS, litterbags were placed under two and in the MCS under three cacao trees. Eight cacao and eight erythrina litterbags were placed at each tree, for the eight sampling dates during one year. Litterbags were removed at eight time points (after 15, 45, 76, 107, 166, 288, 319 and 349 days after placing). Three hundred and twenty litterbags of each litter type were distributed in the plots in total.

Additionally, a part of the fresh cacao and erythrina leaves of each plot (approx. 20 g each sample) were dried at 70° C until constant weight as a reference and correction-factor for the dry weight (Cotrufo et al., 2010). When litterbags were removed from the plots, the litter was weighed, dried until constant weight at 70° C and weighed again, to estimate the mass loss (Olson, 1963; Bärlocher, 2005).

3.3.4. Data analysis

The statistical analyses were carried out with R (R-3.6.1) and RStudio (1.2.5019) (R Core Team 2015). Further, the software packages 'lme4' (Bates et al., 2015) and 'lmerTest' (Kuznetsova et al. 2017) were used. Linear mixed-effects models were applied for SOC, N, Cmic and Nmic. Crop diversity (MCS and AFS) and management practices (conv and org) entered the model as fixed factors, as did their interaction. The four repetition blocks were considered as random factors. Each soil horizon was analysed separately. Datasets showing differences were subjected to post-hoc analyses to know the direction of the effect. For this purpose, pairwise comparisons of least square means (LSMeans) using the 'lsmeans' function of the 'lsmeans' package (Lenth and Hervé 2015) were done. The significance level was set to $\alpha=0.05$.

Leaf litter mass loss was calculated as percentage of original dry mass. The decomposition rate (k) was estimated with the single exponential decay model after Bärlocher 2005:

$$\ln(L_t/L_0)=-kt$$

where L_t is the litter mass recovered from bags at time t ; L_0 is the initial litter mass; k is the exponential decay coefficient; and t is time in days. The single exponential model is commonly used to describe decomposition processes in comparing differences between treatments (Harmon et al., 1999; Moorhead and Sinsabaugh, 2006; Castanho et al., 2012). Exponential models for each leave type and production system were generated.

The R package “ggplot2” version 3.2.1 was used for producing graphs (Wickham, 2016). A single factor ANOVA was also performed for each sampling time and leaf type. In the case that differences were significant, post-hoc analyses were performed through multiple t-tests between the values of the different systems.

3.4. Results

3.4.1. Soil carbon and nitrogen

The A-horizon had an average thickness of 9 cm (between 8 and 11 cm) in all plots. The SOC stocks per hectare in the A-horizon under cacao trees were higher in org compared to conv management practices, while there are no significant differences between the MCS and AFS with the same management practices (Figure 8 and Table 2). The SOC stocks for MCS were on average 33 and 38 Mg ha⁻¹ and in the AFS sites were 29 and 39 Mg ha⁻¹, conv and org, respectively. For the C concentration in the A-horizon, the differences between the org and the conv system were significant. The concentration was 22% (MCS) and 29% (AFS) higher in the organically-managed systems than in conv managed systems.

The SOC stocks for the B-horizon were significantly lower than in the A-horizon, and there is no statistic difference between the systems, although there was a trend towards higher values in organically managed plots (F-value = 3.49, p≤0.10; Table 2). The average SOC stock was 22 Mg ha⁻¹, with the highest stock of 26 Mg ha⁻¹ in the MCS org. Similar to the SOC stock in the B-horizon, the C concentration showed no differences between the production systems.

Among the erythrina trees, a clear distinction of SOC stocks can be seen between the conv (31 Mg ha⁻¹) and org (39 Mg ha⁻¹) plots in the A-horizon (Figure 8). No differences between the systems were recorded for the B-horizon. However, the stocks did not differ on average from the values of the cacao trees of the corresponding management. Statistically, there were also no differences in the C concentration under the erythrina trees, neither in the A nor the B-horizon.

Management practice significantly affected N contents. In the systems with org management, the nitrogen stocks were on average 4 Mg ha⁻¹ and 4.2 Mg ha⁻¹, whereas in the conventionally managed systems the amounts were on average 3.4 Mg ha⁻¹ and

3.2 Mg ha⁻¹, for the MCS and AFS respectively. Total soil nitrogen under the erythrina trees show similar tendency between conv (3.4 Mg ha⁻¹) and org (4.5 Mg ha⁻¹) AFS. No effects on N were found for the B-horizon.

The C/N ratio ranges from 8.7 in the AFS org under the erythrina trees (A-horizon) to 10.1 in the MCS org under cacao (B horizon), but with no statistical differences were found either between the systems, the trees or between the A and B horizons.

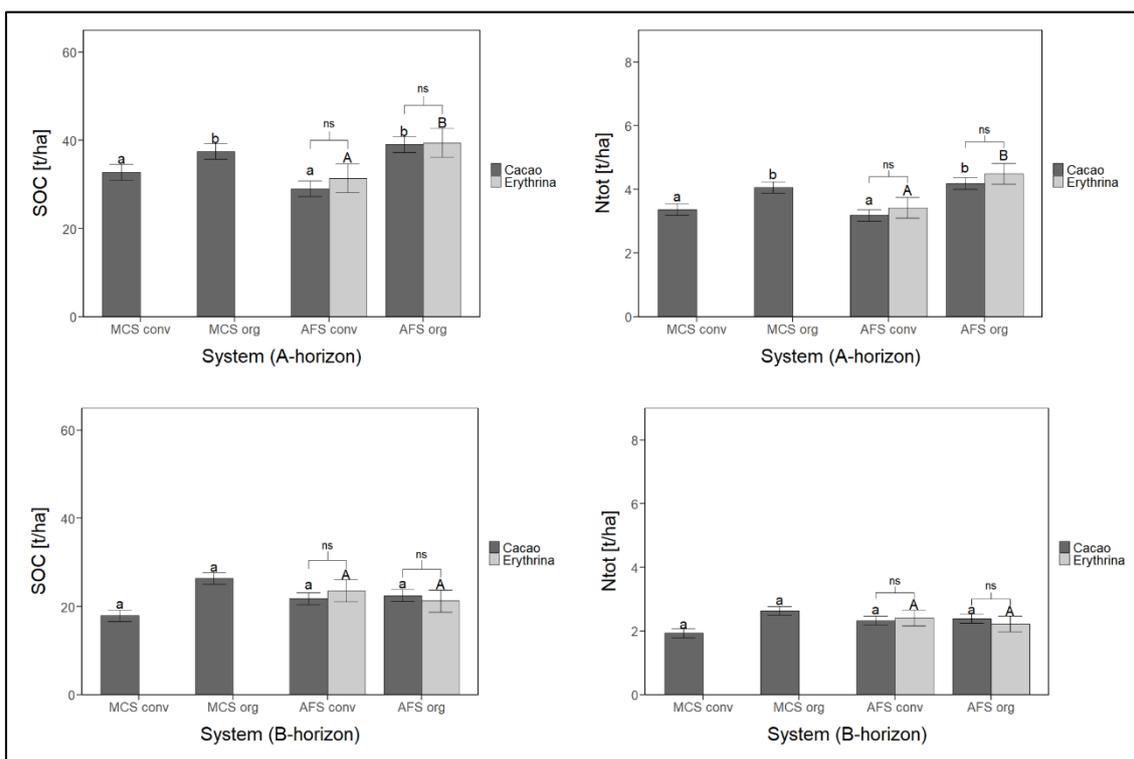


Figure 8: Soil organic carbon stocks (SOC) and total Nitrogen (Ntot) for two soil layers (A-horizon and B-horizon) of the four different cacao production systems for 2014.

Bars represent the mean of four repetitions for each of the four different cacao production systems (MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management). Means and standard errors presented are non-transformed data. Lower case letters indicate significant differences ($p \leq 0.05$) between the cacao trees. Differences between *Erythrina* trees are indicated with upper case letters, while differences between cacao and *Erythrina* trees in the AFS are indicated with ns (no significant) and * (significant; $p \leq 0.05$).

3.4.2. Microbial carbon and nitrogen

Cmic strongly decreased from the O-horizon to the subsoil in all systems (Figure 9). The largest statistical differences between the Cmic concentrations were found in the surface layer O. In the case of cacao trees, differences in Cmic concentrations became apparent, both between the different management practices and between the different cropping systems. The lowest value of Cmic concentrations was found for MCS conv (393 µg/g) and the highest value for AFS org (1375 µg/g).

There were no significant differences in the A-horizon in Cmic concentrations, neither between cacao nor between erythrina trees (Table 3). Also there were no significant differences in Cmic concentrations between cacao and erythrina trees in the AFS. There was a significant difference in AFS org in Cmic concentrations, with higher concentrations under *Erythrina* trees compared to cacao trees. The concentration in the B-horizon ranges from 80 to 107µg/g. There were no significant differences in the Cmic concentrations between the erythrina trees and no differences between cacao and erythrina trees within the same management practice.

Microbial nitrogen concentrations under cacao trees in the O-horizon were four times higher in the organically managed systems than in the conv systems (Figure 9). With 230 µg/g of microbial nitrogen, the AFS org had the highest concentrations of microbial nitrogen. In the O-horizon the range of values of Nmic between conv and org MCS was largest (28 µg/g and 67 µg/g), while the values for AFS were not significantly different (50µg/g and 48µg/g). In the B-horizon no differences between the individual systems could be identified. The concentrations for the erythrina trees were statistically indifferent for both AFS management practices. There was a significant decrease from the surface layer (conv: 221 µg/g; org: 335 µg/g) to the subsoil (conv: 22 µg/g; org 18 µg/g). Differences of microbial nitrogen between cacao and erythrina trees could be found both in the surface layer in the AFS conv and in the topsoil in the AFS org. The microbial nitrogen concentration in the B-horizon also differed between cacao and erythrina trees in both AFS. The direction of the effect was the same in all cases, with higher values under the erythrina trees.

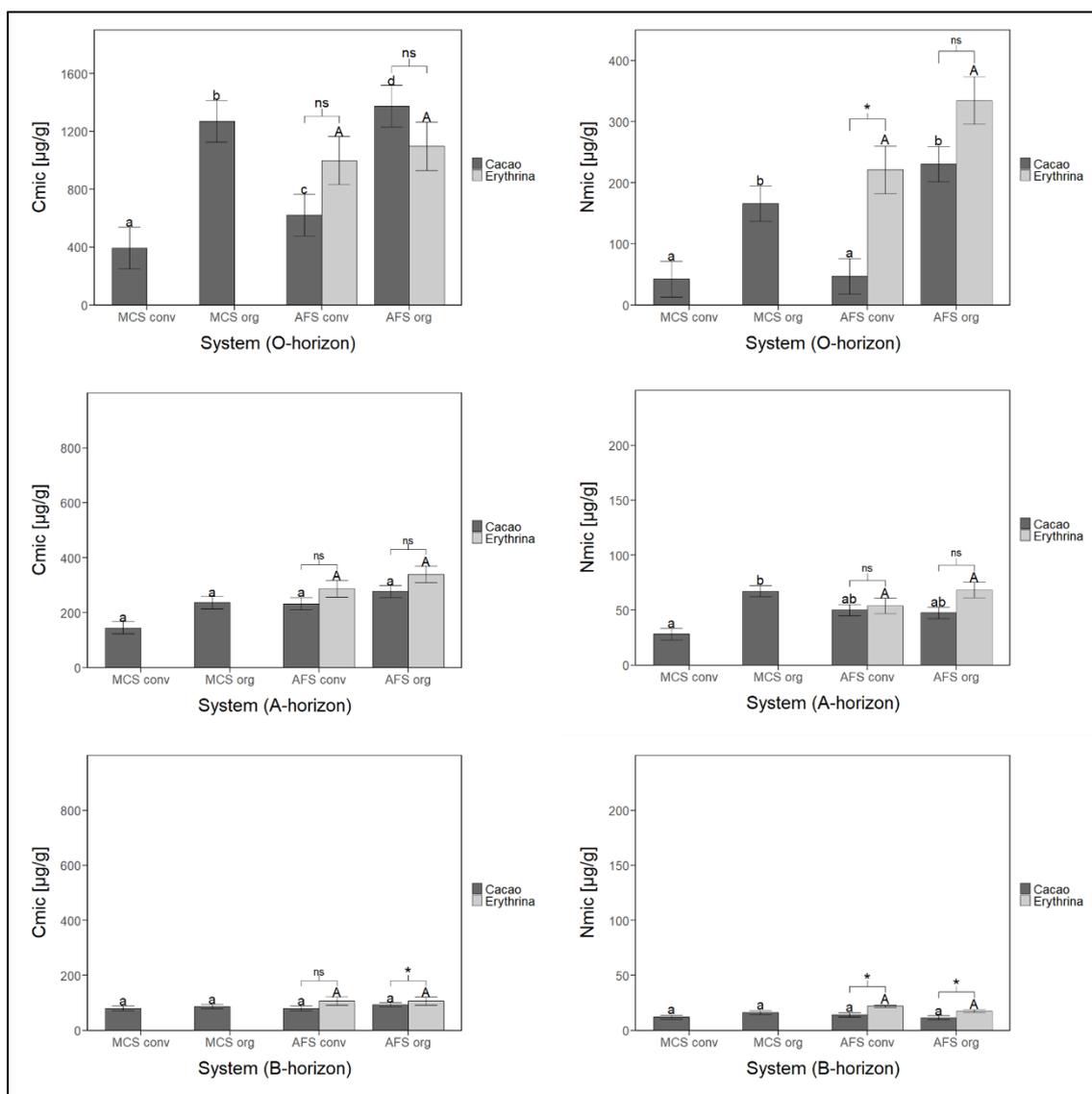


Figure 9: Microbial carbon (Cmic) and microbial nitrogen (Nmic) for three soil layer (O-horizon, A-horizon and B-horizon) of the four different cacao production systems for 2014. Bars represent the mean of four repetitions for each of the four different cacao production systems (MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management). Means and standard errors presented are non-transformed data. Lower case letters indicate significant differences ($p \leq 0.05$) between the cacao trees. Differences between *Erythrina* trees are indicated with upper case letters, while differences between cacao and *Erythrina* trees in the AFS are indicated with ns (no significant) and * (significant; $p \leq 0.05$).

Table 2: Results of the linear mixed models for Soil organic carbon (SOC), carbon content (C %), total soil nitrogen (N total), nitrogen content (N%) and the carbon nitrogen ratio (C/N) under cacao trees and *Erythrina* trees, and the difference between both tree types for four different cacao production systems.

	Cacao trees				<i>Erythrina</i> trees					
	Crop diversity		Management		Management		AFS conventional		AFS organic	
	F value	Direction of the effect	F value	Direction of the effect	F value	Direction of the effect	F value	Direction of the effect	F value	Direction of the effect
SOC [t ha ⁻¹]	A-Horizon	0.104	5.01*	org > conv	0.64		12.34*	org > conv	1.33	0.00
	B-Horizon	0.0001	3.49 .	org > conv	2.52		0.13		2.02	3.25
C %	A-Horizon	0.0291	10.63**	org > conv	0.22		0.55		2.12	0.73
	B-Horizon	0.1951	2.84		0.73		0.01		1.13	0.27
N total [t ha ⁻¹]	A-Horizon	0.0059	8.03*	org > conv	0.25		12.52*	org > conv	0.97	0.44
	B-Horizon	0.0589	2.38		1.25		0.04		1.81	2.46
N %	A-Horizon	0.0027	11.21**	org > conv	0.00		0.91		1.96	0.11
	B-Horizon	0.0682	1.71		0.27		0.02		1.00	0.00
C/N	A-Horizon	1.1155	0.14		1.69		1.21		1.31	1.31
	B-Horizon	1.0836	1.16		1.42		0.05		0.59	0.59

. p≤0.10; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

Table 3: Results of the linear mixed models for microbial Carbon (C mic) and Nitrogen (N mic) under cacao trees and *Erythrina* trees, and the difference between both tree types for four different cacao production systems.

	Cacao trees			<i>Erythrina</i> trees		Difference cacao and <i>Erythrina</i> trees		
	Crop diversity	Management	Crop diversity×Management	Management	Direction of the effect	AFS conventional	Direction of the effect	AFS organic
	F value	Direction of the effect	F value	Direction of the effect	F value	Direction of the effect	F value	Direction of the effect
C mic	5.73 *	AFS >MCS	54.31 ***	org > conv	0.33	0.54	0.63	
[µg/g]	4.16 .	AFS >MCS	4.77 .	org > conv	0.70	3.24	1.51	
	0.04		0.65		<0.01	1.25	13.59 *	E > C
N mic	5.51 .	AFS >MCS	67.13 ***	org > conv	2.92	14.95 *	2.62	
[µg/g]	0.38		6.78 *	org > conv	0.99	0.31	9.81 .	E > C
	0.03		0.28		8.28 .	9.27 *	7.55 *	E > C
					conv > org	E > C	E > C	E > C

. p≤0.10; * p ≤ 0.05; ** p ≤ 0.01; *** p ≤ 0.001.

3.4.3. Leaf litter decay rates of Cacao and erythrina leaves

The leaf litter decomposition curves of cacao ($R^2=0.83$) and erythrina ($R^2=0.83$) leaves corresponded well with the chosen single exponential decay model suggested by Bärlocher 2005 (Figure 10). There were no significant differences in the decomposition of cacao leaves between the different systems at the different sampling times, except for the last sampling, after 349 days. The decomposition of cacao litter in the AFS org plots was significantly different from the three other systems. The distribution pattern of erythrina leaf litter decomposition did not differ between production systems at any sampling time.

The exponential decay coefficient for cacao leaf litter was 0.002/day. During the first 20 days, about 8% of the original cacao leaf litter had disappeared. After 120 days, two thirds of the mass of the cacao leaves had not yet decomposed. The half-life (50% remaining mass) of the cacao leaf litter was 325 days.

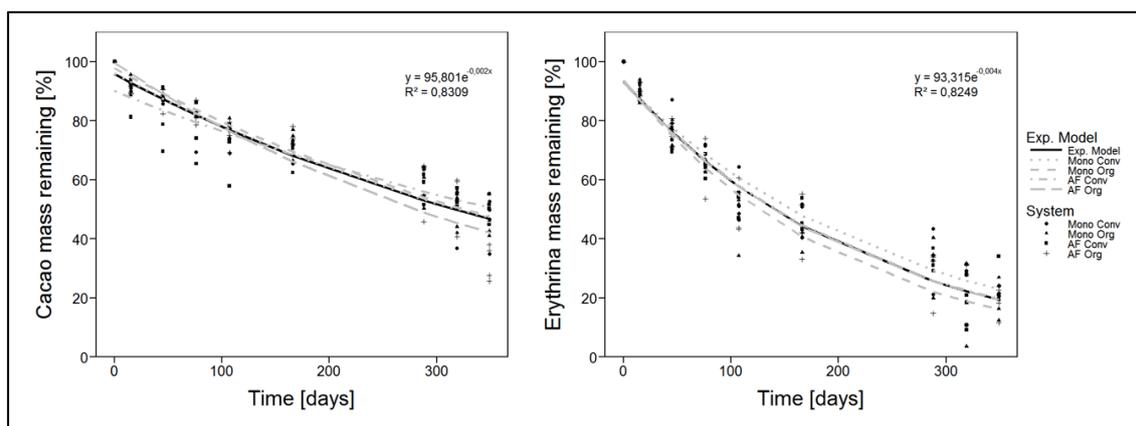


Figure 10: Leaf litter decomposition of cacao and *Erythrina* in four different cacao production systems.

(MCS conv = Monoculture systems under conventional management, MCS org = Monoculture systems under organic management, AFS conv = Agroforestry systems under conventional management, AFS org = Agroforestry systems under organic management). Lines represent the exponential decay model for each production system. The solid bold line and the statistical values represent the exponential model within all systems. The exponent represents the daily decomposition rate. The different dots represent the mean values of the different plots.

The mass loss of erythrina leaf litter within the first 20 days was 14%. The decay coefficient for erythrina leaf litter was 0.004/day. By the end of 120 days, 58% of the original leaf litter mass remained in the litter bags. The half-life of erythrina leaves was 156 days.

3.5. Discussion

3.5.1. Soil Carbon and Nitrogen in monoculture systems (MCS) and agroforestry systems (AFS)

Due to the expected great heterogeneity in soil nutrients, especially in forest systems (Schneidewind, 2011; Schöning et al., 2006), the focus in this study was placed on only two components in the production systems. On the one hand, SOC and N contents, as influenced by the incorporation of legume trees, were determined by comparing soil samples from vicinity of cacao and erythrina trees in the AFS. On the other hand, in contrast to previous studies, the soil samples were taken on a horizon-related basis in order to track changes within the depth profile on a process-related base. Differences in SOC and nitrogen concentrations were only found for the topsoil. Contrary to the expectations, there were only differences between organically and conv management practices, but no significant differences between AFS and MCS of the same management. This includes both the cacao trees and the erythrina trees. A trend towards higher total concentrations of C and N in org systems was also observed by Payan Zelaya (2005). The higher stocks in the org systems can be directly related to the compost application under the cacao trees, which contrary to mineral fertilisers, contribute to build up the organic matter pool.

Another factor that could have contributed to the higher C and N pools is the increased root density in organically-managed systems due to the presence of the perennial leguminous cover crop (Niether et al., 2018). The leguminous ground cover crop spread over the entire org plots (MCS and AFS) and were only systematically removed from around the cacao stems. Niether et al. (2018) showed a much higher root density in the MCS org than in any other system and that 80% of the root turnover takes place within the first 25 cm. The dense root system in org managed systems lead therefore to increased C and nitrogen stocks. Precisely because the two org farming systems had similar high SOC stocks in the topsoil suggests that the root system plays an essential role in the C and nitrogen accumulation. A high root turnover and the importance for the C input in AFS are also highlighted by Hertel et al. (2009). In arable systems, a global meta-analysis also showed a more pronounced effect of org management practice on topsoil C stock accounting for $\sim 3.50 \text{ Mg C ha}^{-1}$ (Gattinger et al., 2012).

In seven different farming systems in Brazil, Monroe et al. (2016) also found significant differences in the topsoil (0-20 cm), with young AFS and pasture showing the highest C levels. They found up to 44% of SOC stock in the first 20 cm. In the upper 10 cm of a 4-year-old cacao and rubber AFS, Monroe et al. (2016) determined 57 Mg C ha⁻¹, which is almost 1.5 times higher than in the org AFS in Sara Ana. They likewise see the root systems of these newly established AFS and the plant residues from the pruning of the cover crop and banana as the source of the C content. Norgrove and Hauser (2013) indicated only 15 Mg C ha⁻¹ in the upper 10 cm in a 35-year-old AFS in Cameroon. Isaac et al. (2005) found 22.6 Mg C ha⁻¹ in the upper 15 cm in 2-year-old multistrata agroforestry in Ghana. Within a chronological sequence the authors showed that the SOC stocks in AFS have reached a steady state within 15 years, with lower values than in the initial phase. The authors attributed the higher SOC values at the beginning to land preparation methods. Considering that the systems in Sara Ana were not even six years old, it is therefore to be expected that the build-up of the humus layer is not yet completed and raising C and nitrogen levels might be expected in the coming years.

As a result of the equal distribution of C and nitrogen values, the C/N values in the different systems do not differ. A C/N ratio between 9 and 10 corresponds to the values observed for most tropical soils (Condrón et al., 1990, Zaia et al., 2012) and indicates a balanced budget and rapid nutrient turnover.

Different C and N stocks between cacao and erythrina samples were expected, due to the functional property of the erythrina trees to fix nitrogen. The legume tree introduces therefore a considerable amount of C and nitrogen into the system through pruning and leaf-litter fall (Schneidewind et al., 2019). Measured nutrient contents in the biomass of erythrina species are sometimes quite significantly higher than those of cacao (Beer et al., 1990; Isaac et al., 2007; Schneidewind et al., 2019). Hartemink (2005) points out that erythrina trees have a strong influence on the nitrogen content of the soil. Soils in AFS in Costa Rica with erythrina poeppigiana had about 1 Mg ha⁻¹ more nitrogen than soils under non-leguminous shade trees. Hagggar et al. (2011) showed also a positive effect on nitrogen mineralization of moderately pruned erythrina trees, related to the higher levels of biomass recycling and nitrogen fixation. That shade trees can have little influence on the plot scale, but localized positive effects on important soil health

parameters is pointed out at Blaser et al. (2017).

In addition, the pruning residues of the erythrina and cacao trees were not distributed in the area, but were placed in the area under the cacao crown. So that a general horizontal exchange of nutrients takes place system-wide. Payan Zelaya (2005) found higher C and N concentrations in surface soil near erythrina trees in conv farming systems, but found no evidence of this effect in org farming systems. The author attributed this also to the even distribution of biomass in organically managed systems. Furthermore, the erythrina trees in Sara Ana were at a distance of 4 metres from the cacao trees and the root network of both the cacao trees and the erythrina trees overlapped (Niether et al. 2018). With more than 4 Mg N ha⁻¹ in the upper 25 cm, even in conv MCS, the nitrogen stocks in this study are far above those from Ghana reported by Isaac et al. (2005). However, the values in the AFS in this study are still in the lower third of the values for cacao AFS described by Hartemink (2005). This may be due to the fact that the systems were, relatively young (6 years). The nitrogen present in cacao agroecosystems is to be found in the topsoil, and the contents in the first 30 cm range from 4 to 19 Mg ha⁻¹.

3.5.2. Soil microbial Carbon and Nitrogen in cacao monoculture systems (MCS) and agroforestry systems (AFS)

Microbial biomass changes much faster than SOC and nitrogen pools. Therefore, the microbial nutrient contents are a good early indicator for changes in cultivation methods and interventions in the ecosystem (Fließbach and Mäder 2000; Powlson et al. 1987). The microbial concentration in the O-horizon were higher in AFS than in monocultures and in organically managed systems higher than in conv ones. This also includes the A-horizon, but only for the microbial nitrogen. It was expected that there would be little or no differences in the B-horizon, since microbial activity takes place mainly in the upper centimetres.

In their 2011 study, Alfaro-Flores et al. (2015) found differences between AFS and monocultures, but could not prove a statistical difference between conv and org management in Sara Ana. This can be explained by the fact that pooled samples were taken at different distances from the cacao trees and that no differentiation of soil horizons was made. Another factor is, that at the time of the study of Alfaro-Flores et al.

(2015), the systems were three years younger and less compost was applied up to this year. Therefore, the differentiation between production systems were less pronounced. However, on average, the concentrations for the A-horizon were of the same order of magnitude, while the concentrations for the mineral B-horizon were much lower. The concentrations given by Zaia et al. (2012) for cacao AFS are also in the same range. That a greater difference can be found with higher values in arable org managed systems due to long-term management was shown by Fließbach and Mäder (2000) and also in Lori et al. (2017).

In addition to most previous studies, in this study not only the topsoil (A-horizon) was analysed for microbial activity, but also the organic layer (O-horizon). This gives a clearer picture, that soil microbial biomass values were found to be up to 5 times higher in organically managed systems than in conv systems.

The microbial activity in conv monocultures hardly differed between the O-horizon and the A-horizon. In conv monocultures only a small amount of biomass is available, with pruning residues and litter-fall from cacao as main source (Schneidewind et al., 2019). While in AFS, fresh material for decomposition is constantly produced by litter-fall and pruning residues (Beer et al., 1990; Schneidewind et al., 2019; Zaia et al., 2012). The general lower level of microbial activity in the conv systems is due to the generally lower C levels, but may also be due to the regular application and toxicity of the herbicides (García-Orenes et al., 2010). With regard to microbial nitrogen values in the organic cover layer, it is most noticeable that among conventionally managed cacao trees the value is five times lower than to the organically managed cacao trees. The value is so low in comparison and at the level of the topsoil that it seems that there is no developed organic surface layer among conv cacao trees due to the lack of compost. In organically managed systems also the high root density due to the ground cover (Niether et al., 2018) and the applied compost are biomass sources that explain the increased microbial activity (Araújo et al., 2008).

The fact that there are no significant differences in the microbial biomass between the erythrina trees in organically and conventionally managed plots was not surprising, since there is no difference in the specific management of erythrina trees. However, there are significant differences between the cacao and erythrina trees. These differences occur

mainly in the B-horizon. Both the Cmic and Nmic concentrations are higher for the erythrina trees. This can be explained by the root structure of the different trees. The cacao trees have a deep reaching tap root on the one hand and a dense fine root network on the soil surface on the other. The erythrina trees have both superficial roots and a deep reaching root network, which spreads laterally. The fact that erythrina trees form a dense root network in the upper soil horizons is also described by Chesney and Nygren (2002). The legume-rhizobia symbiosis of the erythrina roots can explain the higher values of microbial nitrogen in the B-horizon. Thus, we interpret higher Nmic concentrations below the A-horizon as an early indication for increasing N concentrations in the future.

3.5.3. Litter decomposition

Conventional agricultural systems are highly dependent on fertilizer applications. Low input agriculture systems maintain sustainable through external nutrient inputs, like dry and wet deposition, an internal nutrient cycle, and the fertility of the soil. Diversified AFS support low-input agriculture through biomass by litter fall and pruning. Nutrients and organic matter bound in the AGB are thus returned to the soil. In systems with perennial plants, nutrient recycling can be accelerated and enhanced by regular tree pruning (Schneidewind et al., 2019). Litter and pruning residues are a central nutrient resource in AFS and an important link between plants and soils for the return and recycling of organic matter and nutrients (Hartemink, 2005; Triadiati et al., 2011). After the addition of fresh plant residues to the soil surface, drying and wetting cycles as well as temperature have an important influence on microbial activity and biodiversity (Cabrera et al., 2005). In addition to abiotic and biotic factors, the chemical composition of the litter and pruning residues plays a significant role in biomass decomposition (Prescott, 2010). The faster the biological decomposition, the faster nutrients are released and available to the system. The decomposition of organic material and mineralisation processes are therefore essential for the uptake of nutrients by plants. On the other hand, a slower decomposition helps to build up an organic layer and to sequester C in the topsoil. Therefore, plant decomposition residues improve soil quality in many ways with positive effects, on bulk density and soil structure, infiltration and water holding capacity, erosion, and soil nutrient retention (Bünemann et al. 2018;

Murphy 2014).

In this study remarkable differences between the daily mass decay rate of cacao leaves and erythrina leaves were found. The decomposition of erythrina leaves is almost twice as fast as that of cacao leaves. Nutrients stored in erythrina leaves are thus released much faster and are available to the system more quickly and help to meet plant need for nutrients. In contrast, the cacao leaves, due to their slower decomposition and higher levels of lignin and polyphenol, help build up an organic topsoil layer and nutrient stocks in soil (Dawoe et al., 2010; Isaac and Nair, 2005; Schneidewind et al., 2019) and serve to protect the soil structure. The daily rate of decomposition of the cacao leaves in this study reflects the results obtained by Muoghalu and Odiwe (2011) in Nigeria and also the results found in a laboratory experiment by Mohammed et al. (2019). According to the classification given by Bärlocher (2005), the decomposition of cacao leaves is slow and that of erythrina leaves is medium. The rate of decomposition appears to be relatively slow compared to field observations. This may be due to the fact that in this experiment the litterbags were made of stable nylon material, since in previous experiments the litterbags were made of finer not so stable tissue and were destroyed by insects in a few days. The selected mesh size may have therefore a strong influence on the decomposition. The selected mesh size of 1 mm excludes the macrofauna, which is responsible for the first decomposition steps and was seen by Lavelle et al. (1993) as an important factor in the regulation of decomposition in the humid tropics. By excluding macrofauna, it was expected that greater differences between systems would occur.

However, there are no differences between org and conventionally managed systems in this study, where decomposition was expected to be faster due to a supposedly higher microbial activity (Prescott, 2010). Asigbaase et al. (2021) on the other hand, was able to demonstrate this difference between conv and org cacao AFS systems. That the differences were so pronounced may be because the farms selected were between 20 and 30 years old. That management practices, such as the use of herbicides and pesticides, have a negative impact on decomposition is also assumed by Muoghalu and Odiwe (1992). Also through microclimate factors, no differences occurred between MCS and AFS. Saj et al. (2021) also made the same observation that leaf-litter decomposition

could not be directly correlated to any of the microclimatic data. In contrast, Seidelmann et al. (2016) ascribed 31% of the variance in decomposition to microclimatic conditions. Monocultures have a microclimate with lower soil moisture, as it was observed in the conv MCS in the Sara Ana trial (Niether et al., 2018). The AFS on the other hand buffer extreme climate events, which leads to a constant higher moisture in AFS (Niether et al. 2017). The fact that even under these conditions no notable differences between MCS and AFS can be observed in decomposition rates, is astonishing. Chemical composition of the leaves, such as polyphenols concentration, nitrogen and lignin content and the C/N ratio (Berendse et al., 1987, Palm et al., 2001) seems to play a greater role in decomposition in cacao production systems than the microclimate. The differences shown in decomposition and nitrogen concentrations (Schneidewind et al., 2019) between the two leaf types therefore allow recommendations to be made for the soil fertility management of cacao AFS. Palm et al. (2001) presented four different management categories of organic resources, depending on nitrogen and lignin content. According to this classification, cacao leaves, with low nitrogen content, can be used well for erosion control and water balance regulation, while the nitrogen-rich erythrina leaves can be used for the direct incorporation of nitrogen in the soil biome. With the high inputs from litterfall and pruning of cacao trees and legumes (Schneidewind et al., 2019; Zaia et al., 2012), soil fertility can be maintained or improved in the long term even without the external input of industrial fertiliser.

3.6. Conclusions

AFS with diverse trees and specific functions are predestined for production without external input from agrochemicals. Six years after the installation of the experimental plots in Sara Ana, higher SOC levels are shown in organically managed production systems compared to conv managed cacao production systems. Microbial biomass in organically managed systems is consistently higher in the topsoil than in conv cacao production systems. So far, the characteristics of the different systems have affected only the topsoil. Differences in the subsoil have only been in microbial biomass with higher values under the erythrina trees compared to the cacao trees. Incorporating leguminous trees into AFS is a source of easily decomposable and nitrogen-rich litter, while residues of cacao trees help to build up SOC stocks. Organically managed cacao

AFS lead to healthier soils and should therefore form the basis for production that is not only designed for short-term profit, but also preserves soil ecosystem services in the long term.

Contributions of authors

Conception and design of the study: Ulf Schneidewind, Florian Hackmann, Felix Heitkamp and Gerhard Gerold. Material preparation, data collection and analysis were performed by Ulf Schneidewind, Florian Hackmann, Felix Heitkamp and Laura Armengot. The first draft of the manuscript was written by Ulf Schneidewind and all authors commented on previous versions of the manuscript.

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4. Below- and aboveground production in cacao monocultures and agroforestry systems



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Below- and aboveground production in cacao monocultures and agroforestry systems

4.1. Abstract

Farmers expect yield reduction of cash crops like cocoa when growing in AFS compared to MCS, due to competition for resources, e.g. nutrients and water. However, complementarities between species in the use of resources may improve resource use efficiency and result in higher system performance. Cacao trees have a shallow rooting system while the rooting characteristics of the associated trees are mainly unknown. This work investigates fine root distribution and production in five cacao production systems: two MCS and two AFS under conv and org farming, and a SAFS. In the org systems a perennial leguminous cover crop was planted and compost was added, while herbicides and chemical fertilizers were applied in the conv ones. We measured cacao fine root parameters in the top 10 cm of soil and annual total fine root production at 0–25 and 25–50 cm depth. We related the root data with both the aboveground performance (tree and herbaceous biomass), and the cacao and system yields.

Cacao fine roots were homogenously distributed over the plot area. Around 80% of the total fine roots were located in the upper 25 cm of soil. The total fine root production was 4-times higher in the AFS and the org MCS than in the conv MCS.

The roots of the associated tree species were located in the same soil space as the cacao roots and, in principle, competed for the same soil resources. The cocoa yield was lower in the AFS, but the additional crops generated a higher system yield and AGB than the conv cacao MCS, implying effective resource exploitation. The leguminous cover crop in the org MCS competed with the cacao trees for nutrients, which may explain the lower cocoa yield in this system in contrast with the conv MCS.

4.2. Introduction

Cocoa bean production is a source of income generation for >5 million small-scale famers (Hütz-Adams et al., 2016). It has the potential to contribute to biodiversity conservation in tropical areas (Bisseleua et al., 2009; Tschardt et al., 2011), as well as to C sequestration when cacao trees are grown in AFS, i.e. associated with fruit, timber and/or shade trees (Saj et al., 2017; Schneidewind et al., 2019). The cacao tree

(*Theobroma cacao* L.) is native of the understory of the Amazonian rainforest, and grows well under a shade tree canopy, therefore also in various AFS (Schroth et al., 2004). New cacao varieties are adapted to produce high yields in full-sun monocultures, but they require much higher fertilizer and pesticide inputs (Hütz-Adams et al., 2016).

All the different components of AFS –cash crops, associated trees and herbaceous plants– are continuously interacting. Their interactions are determined by the management of the system, including the selection of species and their functional characteristics, planting density, stratification, and fertilization regime. Aboveground interactions include the exposure of the different species in the various strata of the AFS to climate and weather (Niether et al., 2018). While some shade in AFS can even support the physiological functioning of the cacao trees (Baligar et al., 2008) and their longevity by reducing stressful environmental conditions (Läderach et al., 2013), heavy shade reduces light for photosynthesis and can lead to delayed growth and reduced cocoa yield (Schneider et al., 2017). Belowground interactions in AFS are less obvious than those aboveground, and remain largely unclear and unexplored. In general, the benefits of AFS include the increase in soil organic matter content, the improvement of the infiltration rate and the enhancement of nutrient recycling (Tscharntke et al., 2011).

All roots in the system take up available nutrients and water from the soil. Consequently, interspecies competition for resources is quite likely when roots of different species cover the same soil space, while complementarity occurs when shallow and deep rooting systems are combined to benefit from a greater utilization of resources in a vertical distribution (Ong et al., 1991; Schroth et al., 2001). Root competition may also benefit the cash crop when toxic compounds like cadmium are distributed among the associated species (Gramlich et al., 2017).

Cacao trees have one taproot and a shallow system of lateral roots spreading horizontally around the stem in the topsoil (Abou Rajab et al., 2018; Kummerow et al., 1982). Fertilizers are usually applied in a circle close to the stem to enable fast uptake by lateral roots, which might influence the cacao root growth at this particular place. However, the lateral rooting system is supposed to reach several meters across (personal communication and Nygren et al., 2013), allowing interactions between neighboring cacao trees, but also with species that are planted in between the cacao

tree rows or evenly across the area like a cover crop. Interactions with associated trees in a dense planting pattern are likely, even though knowledge on the horizontal and vertical distribution of the roots of associated tree species is scarce. Thus, information on belowground competition or complementarity in cacao AFS often depends solely on the farmers' observation of the performance of their cash crops (Graefe et al., 2017; Schroth et al., 2001). Little information on rooting depth, segregation and niche allocation in cacao AFS is available (Abou Rajab et al., 2018; Isaac et al., 2014; Kummerow et al., 1982; Schwendenmann et al., 2010), and further knowledge is of high relevance to unravel the potential competitive or complementary root relationships between cacao trees, and between cacao trees and other species in cacao production systems, which might have important implications for the cocoa yield.

Organic crop production highly relies on the interaction between different species, whether through crop rotation or intercropping, resulting in greater crop diversity over space and time (Barbieri et al., 2017). For instance, combining different species helps controlling pests and diseases and improving the nutrient supply, which is of special relevance due to the prohibition to apply synthetic fertilizers and pesticides. Nevertheless, allelopathic effects and competitive interactions are likely to happen. For instance, leguminous trees increase nitrogen availability by N-fixation, but they may compete with the cash crop for other soil nutrients like phosphorous (Lehmann et al., 2000). So far, scarce information on the best org management practices in cacao production systems is available, despite the increasing interest of the main stakeholders for this kind of production system. The same holds true for AFS, since the market for sustainably produced cacao is growing (Lernoud et al., 2017).

The aim of this work is to study cacao roots and the total system root production and their relation with both the cacao and the system production, i.e. the yield of all crops and total standing biomass. We have analysed the horizontal distribution of cacao fine roots, and the total fine root production in five different cacao production systems, including MCS and AFS under org and conv farming. In our experimental design, cacao trees grow in a conv full-sun MCS, supported by mineral fertilizers and herbicides to reduce weeds. In the organically-managed MCS, cacao trees grow together with a leguminous perennial cover crop to reduce weeds and improve nitrogen soil availability,

and compost is also applied. The conv and the org AFS combine cacao trees with various tree species, including banana plants, in high density. Respectively fertilizer and compost are applied but in lower doses than in the MCS. A third SAFS under org management and without any external inputs associates cacao trees with a high density of trees and crops following a semi-natural succession (Niether et al., 2018; Schneider et al., 2017).

We investigated the horizontal expansion of the cacao rooting systems in the top 10 cm of soil and we expected (i) the cacao fine roots to become smaller and less abundant as the distance from the stem increased, and (ii) the cacao fine roots in the organically-managed MCS and AFS to be more abundant than in the conventionally-managed MCS and AFS, to compensate for the lower nutrient availability due to, respectively, the application of compost instead of mineral fertilizers in org systems and the competition with other tree roots in AFS. Additionally, we used ingrowth donuts under the cacao tree crown to measure the total fine root growth and the expected (iii) higher total fine root production in AFS due to higher stem density. We hypothesized (iv) that a higher total fine root production increases competition for soil nutrients, which may be responsible for lower cocoa yields, while the belowground stratification of deep-rooting trees leads to a better exploitation of the soil resources, explaining higher system productions. This is the first study analyzing whole cacao production systems with direct measurements of both the below- and the aboveground production. The latter includes not only the biomass and the cocoa bean production but also the production of other crops.

4.3. Materials and methods

4.3.1. Study site and experimental plot description

The study was conducted at the research site Sara Ana, run by the Research Institute of Organic Agriculture (FiBL) in the region of Alto Beni, in the tropical lowlands of Bolivia, at 15°27'36.60"S and 67°28' 20.65"W, and 380 m a.s.l. The climate is winter dry with an annual precipitation of 1439 mm, 83% relative humidity and a mean temperature of 25.2° C (Niether et al., 2018). The site lies on an alluvial terrace, and the soils are Lixisols and Luvisols (Schneider et al., 2017).

The research site for the long-term trial was established in 2008: five different cacao

production systems were allocated within a randomized complete block design with four repetitions. The cacao production systems comprised full-sun monocultures (MCS) and agroforestry systems (AFS), both under organic (org) and conventional (conv) farming, and a highly diverse successional agroforestry system under org farming (SAFS). The size of each plot was 48 by 48 m (Schneider et al., 2017), and a net plot of 24 by 24 m was defined for data collection.

Cacao trees were planted at a distance of 4 by 4 m in all production systems (625 stems ha^{-1}). In the AFS, banana plants (*Musa* spp.) and various shade trees, including timber, fruit and leguminous trees, were additionally planted in between the cacao rows (Figure 1A). The stem density of banana plants was 866 stems ha^{-1} in both AFS and 634 stems ha^{-1} in the SAFS; there were also 312 stems ha^{-1} of associated woody trees in the AFS and 2708 stems ha^{-1} in the SAFS. A complete list of the agroforest tree species, as well as information on the org and conv managements implemented, are provided in Schneider et al. (2017). Conventional management refers to the application of mineral fertilizers (18–12–24–4 kg Nal-P₂O₅-K₂O-MgO ha^{-1}) around the cacao stems at a distance of approximately 1.2m; herbicides were applied according to requirements, usually about 4 to 5 times per year. No chemical input was used in the organically-managed plots, but compost (24–17–20–18 kg Nal-P₂O₅-K₂O-MgO ha^{-1}) was applied once a year around the cacao stems, and a perennial soybean (*Neonotonia wightii* [Wight & Arn.] J.A. Lackey) was sown as a leguminous cover crop (Figure 11B). Pest and disease management was in all cases performed according to good management practices concerning cacao and shade tree pruning, regular cut-off of diseased fruits and biweekly harvests. No preventive and curative plant protection sprays were applied. The fertilization rate in AFS involved half of the dose used in MCS.

The soil was sampled in November 2015 across the 0 to 25 cm layer. Eleven soil cores were sampled following a zig-zag pattern in each net plot, and they were well mixed to obtain a composite sample per plot. The soil samples were air dried and analyzed at the Laboratorio de Calidad Ambiental (Environmental Quality Laboratory) in La Paz, Bolivia, according to the standard procedures of the International Soil Reference and Information Center (ISRIC; van Reeuwijk, 2002): total nitrogen (N) was measured after Kjeldahl-digestion, and the available phosphorous (P) in a citric acid solution; the

exchangeable bases potassium (K_{ex}) and magnesium (Mg_{ex}) were determined by using the ammonium acetate method; pH was measured in an aqueous solution.

4.3.2. Cacao fine root distribution and biomass

Cacao fine roots were sampled in 2015 at three different distances (0.4 m, 1.2 m and 1.7 m) from the cacao tree stem, in two opposite directions and in two trees per plot (Figure 11C). The 1.2 m distance referred to the mean radius of the cacao tree crown and the fertilization line around the stem, while the 0.4 m distance was always below the crown and the 1.7 m distance was always outside of it. At the sampling sites, the topsoil was cleaned from loose litter and branches. Soil cores were sampled with a 5.3 cm-diameter auger down to a 10 cm soil depth. From the total soil volume of 220.6 cm³, roots were extracted by floating (Lauenroth and Whitman, 1971). The finest roots were not considered and roots thicker than 4 mm, dead roots and roots from other species were also removed from the sample. The 4 mm diameter upper limit for cacao fine roots was applied across all production systems in this study as well as in the determination of the total fine root production (see Section 2.3). The samples were oven-dried at 72° C until constant weight was obtained for biomass determination. Pictures of the dried samples were taken (NIKON COOLPIX P520, aperture: F/8.3, exposure: 1/5 s., ISO 80, resolution: 300 dpi) to avoid variation in their size or volume during measurement. Fine root length, volume, surface area and mean diameter were analyzed with “ImageJ” (Schneider et al., 2012) and “IJ_Rhizo” (Pierret et al., 2013), applying the Kimura length correction on root length. The specific root length and specific root area were calculated from the respective biomass. The data obtained here were used for comparison of root parameters between the production systems in this study, but comparisons with data from other studies should be done cautiously because roots were dried before the measurements, which is not common in other studies.

4.3.3. Total annual fine root production

Following Milchunas et al. (2005), we used the modified root ingrowth donut method to estimate belowground net primary fine root production at two depths (0–25 cm and 25–50 cm), thus covering the whole cacao rooting zone (Kummerow et al., 1982). Root ingrowth donuts were placed at a distance of 0.5 m from the cacao stem, under the cacao crown: three per plot in MCS conv, MCS org and SAFS, and two per plot in AFS

conv and AFS org.

The ingrowth donuts were installed in April 2014 by removing soil to a depth of 50 cm cylindrically within an outside diameter of 22 cm (Figure 11D). The surface of the sides (1728 cm²) was lined with a wire gauze with a net size of 0.5 cm that only allowed the ingrowth of fine roots of all species with a diameter of up to 4 mm. A PVC pipe with an outside diameter of 15 cm was placed inside the hole and filled with sand bags. This pipe was divided into an upper and a lower part of 25 cm each. The 7 cm of donut space between the wire gauze and the pipe in the center was filled with soil that had been previously removed from the same space and was now root-free. Roots were sampled after one year, in April 2015. First, the sand bags were taken out of the pipe. Then the upper part of the pipe was removed, and the soil and roots in the donut space down to 25 cm from the soil surface (resulting in a soil volume of 5085 cm³) were carefully cut from the wire gauze with a sharp knife and collected. Afterwards, the lower soil layer inside the cylinder was also removed. Soil and roots from 25 down to 50 cm deep were collected separately. The roots were separated from the soil by using the flotation method (Lauenroth and Whitman, 1971). The total fine root samples were dried at 72° C during three days for biomass determination.



Figure 11: Conventional cacao agroforestry system with banana plants and associated trees (A) and cacao organic monoculture with leguminous cover crop (B); superficial roots of a cacao tree and the first sampling point from the cacao stem (C) and 0.5 m hole for ingrowth-donut before replacing the root-free soil (D).

4.3.4. Cacao and total system standing biomass and yield

In 2015, the yield and biomass of the cacao trees were obtained from the seven-year-old cacao production systems in the net plot. Cacao pods were harvested every fifteen days all year round. The beans were taken off and weighed. Their dry weight with 0% residual moisture was calculated for each plot by multiplying the fresh weight by 0.25 (in accordance with the 0.33 dry bean factor for 8% residual moisture established by Schneider et al., 2017). In the AFS and the SAFS, banana bunches were counted over the year and weighed. Their fresh weight was estimated by applying a 0.85 factor that only includes marketable fruits. To obtain their dry weight, the fresh weight was multiplied by 0.26 (Schneider et al., 2017). In the SAFS, the fresh weight of additional crops harvested over the year, i.e. fruits of peach palm (*Bactris gasipaes*) and araza (*Eugenia stipitata*) and tubers of ginger (*Zingiber officinale*) and turmeric (*Curcuma longa*), was also measured. Their dry weight was computed by multiplying the fresh weight by a 0.465 factor for peach palm (53.5% moisture content, according to Mora-Urpí et al., 1997), 0.056 for araza (94.4% moisture content, according to Borghi Virgolin et al., 2017), and 0.69 for ginger and turmeric (Schneider et al., 2017). To calculate the standing AGB, we used allometric equations for cacao trees, banana plants, woody trees and palm trees, applying, respectively, the cacao stem diameter at 30 cm above the soil, the stem diameter of woody trees and banana plants at breast height (1.30 m), and the height of the palm trees (see Appendix Table A.1 and Schneidewind et al., 2018). We used the AGB (shoot) and the fine root biomass of the cacao trees to calculate a fine root:shoot ratio to compare the different production systems.

Along a diagonal transect of the net plot, we estimated the herbaceous biomass in four 50-by-50-cm squares. All herbaceous plants were cut, dried at 72° C until constant weight was reached, and weighed.

4.3.5. Statistical analyses

We applied linear mixed-effects models using R (RCoreTeam,2017) and lmerTEST (Kuznetsova et al., 2016) to describe the effect of the type of production system, the distance, and their interaction (system:distance) on the response variables of the cacao fine root measurements (length, volume, surface area, mean diameter, and weight). The side of tree nested to individual tree nested to block was used as a random factor. A

second model was implemented to describe the effect of system, depth, and their interaction (system:depth) on the response variables of the total fine root production (ingrowth donuts). The number of the donut nested to block was applied as random factor. The yield and biomass, as well as the fine root:shoot ratio, were analyzed including the system as the only fixed factor in the model. The block was included as a random factor. When necessary, according to the visual inspection of the residual plots, we transformed the data (log, Box-Cox) to meet the normality and homoscedasticity requirements of the residuals. Orthogonal contrasts were fixed a priori to compare the different levels of the production systems, i.e. MCS were compared to AFS (MCS vs AFS), AFS were compared to the SAFS (AFS vs SAFS) and, within MCS and AFS, conv and org managements were also compared (MCS conv vs MCS org and AFS conv vs AFS org). Orthogonal contrasts were also fixed to compare the samples taken at different distances from the stem (0.4 m vs 1.2 m, and 1.2 m vs 1.7 m) in the first model, and at different soil depths (0–25 cm vs 25–50 cm) in the second model.

4.4. Results

4.4.1. Cacao fine root parameters

We did not observe an influence of the distance from the stem or of the interaction between system and distance on any of the analyzed root parameters, including root length density, volume and surface area density, specific root length, specific root area, mean diameter and biomass (Table 4). Neither did the production system have an effect on the root length, surface area, specific root length, specific root area or diameter. However, the root volume and biomass found in the SAFS were higher than those found in the AFS, while the biomass increased from AFS conv to AFS org.

We observed a strong correlation between the results of the biomass measurements and the data obtained from the root parameter analysis with IJ_Rhizo, especially in the case of biomass density, surface area ($r=0.86$, $p < 0.001$) and volume ($r=0.87$, $p < 0.001$). The correlation coefficients for biomass density and length ($r=0.64$, $p < 0.001$) and for the mean diameter ($r=0.4$, $p < 0.001$) were lower but still significant.

Table 4: Mean \pm standard error of cacao fine root (< 4 mm) parameters at three distances from the stem in five cacao production systems.

Distance [m]	System	Length density [cm cm ⁻³]	Specific root length [cm g ⁻¹ cm ⁻³]	Volume [mm ³ cm ⁻³]	Specific root volume [mm ³ g ⁻¹ cm ⁻³]	Surface area density [mm ² cm ⁻³]	Specific root area [mm ² g ⁻¹ cm ⁻³]	Mean diameter [mm]	Biomass density [g cm ⁻³]
0.4	MONO CONV	8.5 \pm 1.9	44.2 \pm 11.6	4.3 \pm 0.8	14.1 \pm 1.4	5.9 \pm 1.0	23.9 \pm 3.8	0.88 \pm 0.09	0.31 \pm 0.06
0.4	MONO ORG	6.4 \pm 1.2	37.0 \pm 6.3	4.2 \pm 0.9	15.5 \pm 0.9	5.0 \pm 1.0	22.1 \pm 2.3	0.94 \pm 0.06	0.24 \pm 0.05
0.4	AF CONV	9.6 \pm 1.6	45.2 \pm 8.0	3.9 \pm 0.7	16.3 \pm 1.7	6.0 \pm 0.9	25.8 \pm 3.6	0.82 \pm 0.06	0.26 \pm 0.06
0.4	AF ORG	7.3 \pm 1.3	24.2 \pm 4.1	4.1 \pm 0.8	11.3 \pm 1.2	5.3 \pm 0.8	16.0 \pm 1.8	0.94 \pm 0.08	0.33 \pm 0.05
0.4	SAFS	11.2 \pm 2.3	30.4 \pm 6.1	6.5 \pm 1.5	16.0 \pm 2.0	8.3 \pm 1.7	21.0 \pm 3.0	0.88 \pm 0.06	0.56 \pm 0.18
0.4	Mean of systems	8.6 \pm 0.8	36.4 \pm 3.5	4.5 \pm 0.4	14.6 \pm 0.7	6.1 \pm 0.5	21.8 \pm 1.4	0.89 \pm 0.03	0.33 \pm 0.04
1.2	MONO CONV	9.5 \pm 1.8	38.6 \pm 3.6	3.9 \pm 0.9	14.1 \pm 0.5	6.0 \pm 1.1	23.2 \pm 1.4	0.81 \pm 0.05	0.30 \pm 0.07
1.2	MONO ORG	11.5 \pm 1.7	51.8 \pm 9.1	5.5 \pm 1.2	16.1 \pm 1.6	7.5 \pm 1.1	28.0 \pm 3.8	0.74 \pm 0.05	0.37 \pm 0.07
1.2	AF CONV	7.2 \pm 1.1	64.1 \pm 19.0	3.1 \pm 0.5	13.8 \pm 2.3	4.8 \pm 0.8	24.1 \pm 5.5	0.79 \pm 0.05	0.22 \pm 0.04
1.2	AF ORG	12.7 \pm 1.5	33.2 \pm 4.0	4.8 \pm 0.7	12.0 \pm 1.1	8.0 \pm 1.0	20.4 \pm 2.1	0.79 \pm 0.03	0.43 \pm 0.06
1.2	SAFS	7.2 \pm 1.0	32.9 \pm 5.7	4.7 \pm 0.8	16.1 \pm 1.3	5.8 \pm 0.9	22.8 \pm 2.5	0.93 \pm 0.05	0.35 \pm 0.07
1.2	Mean of systems	9.6 \pm 0.7	44.4 \pm 4.7	4.4 \pm 0.4	14.5 \pm 0.7	6.4 \pm 0.5	23.8 \pm 1.5	0.81 \pm 0.02	0.33 \pm 0.03
1.7	MONO CONV	7.2 \pm 1.1	40.5 \pm 3.7	2.8 \pm 0.5	14.6 \pm 0.8	4.6 \pm 0.8	24.5 \pm 1.5	0.79 \pm 0.03	0.22 \pm 0.05
1.7	MONO ORG	10.3 \pm 1.5	36.1 \pm 5.1	5.5 \pm 1.1	14.6 \pm 0.5	7.1 \pm 1.1	22.0 \pm 2.0	0.92 \pm 0.07	0.39 \pm 0.08
1.7	AF CONV	7.1 \pm 1.4	45.4 \pm 9.9	3.2 \pm 0.8	16.6 \pm 4.8	4.6 \pm 1.0	25.9 \pm 5.7	0.77 \pm 0.06	0.22 \pm 0.06
1.7	AF ORG	7.5 \pm 1.3	41.0 \pm 6.3	3.3 \pm 0.6	15.3 \pm 2.2	4.9 \pm 0.8	24.9 \pm 3.4	0.82 \pm 0.04	0.27 \pm 0.06
1.7	SAFS	8.6 \pm 1.6	43.1 \pm 9.3	5.0 \pm 1.1	16.3 \pm 2.3	6.4 \pm 1.2	26.8 \pm 4.6	0.91 \pm 0.07	0.40 \pm 0.10
1.7	Mean of systems	8.1 \pm 0.6	41.1 \pm 3.1	4.0 \pm 0.4	15.5 \pm 1.1	5.5 \pm 0.5	24.8 \pm 1.6	0.84 \pm 0.03	0.30 \pm 0.03
Analysis of variance		F value	F value	F value	F value	F value	F value	F value	F value
System		0.5	1.1	2.9	1.7	1.4	0.7	1.9	3.2
Distance		1.5	1.6	0.7	0.1	1.1	1.2	2.1	0.5
System:distance		1.9	0.6	0.7	0.7	1.3	0.7	1	1
Orthogonal contrast		t value	t value	t value	t value	t value	t value	t value	t value
MONO vs AF		0.3	1	n.s.	1	n.s.	1.4	n.s.	-0.5
AF vs SAFS		-0.3	1.1	n.s.	-2.3	*	-1.5	n.s.	-2.4
MONO CONV vs MONO ORG		-0.8	0	n.s.	-1.8	n.s.	-0.9	n.s.	-1
AF CONV vs AF ORG		-1.2	1.7	n.s.	-1.3	n.s.	0.7	n.s.	-2.4
0.4 m vs 1.2 m		-0.7	n.s.	-1.8	0.6	n.s.	-0.2	n.s.	0.3
1.2 m vs 1.7 m		0.9	n.s.	-0.5	1.2	n.s.	-0.5	n.s.	1

MONO CONV: monoculture conventional; MONO ORG: monoculture organic; AF CONV: agroforestry conventional; AF ORG: agroforestry organic; SAFS: successional agroforestry system; F values: analysis of variance; t values: orthogonal contrasts; levels of significance: * p < 0.05; ** p < 0.01; *** p < 0.001; n.s. non-significant.

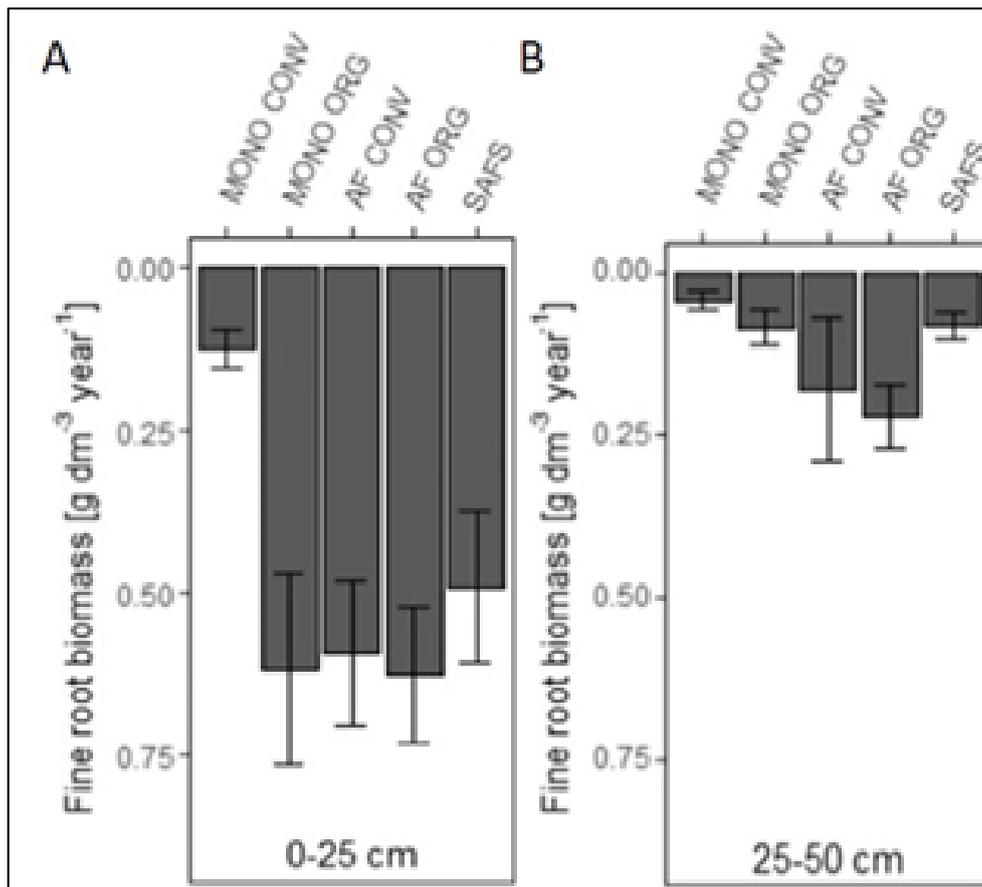


Figure 12: Annual fine root production (mean and standard error of dry weight) at 0.5 m distance from cacao stems at (A) 0 to 25 cm soil depth and (B) 25 to 50 cm soil depth. In five cacao production systems: monoculture conventional (MCS conv) monoculture organic (MCS org), agroforestry conventional (AFS conv), agroforestry organic (AFS org) and successional agroforestry system (SAFS).

4.4.2. Annual fine root production

The total fine root production of all five cacao production systems decreased from the upper to the lower soil layer. No interaction between the type of production system and the depth of the total fine root production was observed (Figure 12, Table 5). The mean annual fine root production did not differ between the AFS and the SAFS, but it was significantly lower in the MCS when compared to the AFS. This was due to the low fine root production in the MCS conv, which was, in addition, significantly lower than in the MCS org. In contrast, no differences were observed in the production of fine roots between the AFS org and the AFS conv. In all production systems, the majority of fine roots were produced in the upper soil layer (Figure 12A) and to a lower amount in the 25–50 cm layer (Figure 12B). The share of fine root production in the upper layer ranged from 74% in the MCS conv and the AFS org to 77% in the AFS conv, 86% in the SAFS and 87% in the MCS org.

Table 5: Results from the linear mixed-effects model for total fine root production in two depths in five cacao production systems.

Analysis of variance	F value	
System	7.8	**
Depth	66.1	***
System : depth	2.1	n.s.
Orthogonal contrast	t value	
MCS vs AFS	-3.4	***
AFS vs SAFS	0.6	n.s.
MCS conv vs MCS org	-3.8	***
AFS conv vs AFS org	-1.3	n.s.
0 - 25 cm vs 25 - 50 cm	8.1	***

MCS conv: monoculture conventional; MCS org: monoculture organic; AFS conv: agroforestry conventional; AFS org: agroforestry organic; SAFS: successional agroforestry system; F values: analysis of variance; t values: orthogonal contrasts; levels of significance: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. non-significant.

4.4.3. Cacao and total system aboveground biomass

The cacao trees growing in the MCS had the highest biomass, compared to those in the AFS and, particularly, those in the SAFS, which had the lowest biomass (Figure 13A, Table 6). No differences in cacao tree biomass were observed between the MCS conv and the MCS org, nor between the AFS conv and the AFS org. The fine root:shoot ratio was lowest in the MCS, as compared to the AFS. Again, there were no differences observed between the MCS conv (0.03 ± 0.01) and the MCS org (0.04 ± 0.01), or between the AFS conv (0.05 ± 0.01) and the AFS org (0.07 ± 0.01). The highest fine root:shoot ratio was found in the SAFS (0.09 ± 0.01).

The associated woody trees and banana plants in the AFS contributed with additional biomass, about 4-times larger than that of the cacao trees (Figure 13A). No differences in total biomass were found between the AFS conv and the AFS org, nor between the AFS and the SAFS. The biomass of the associated trees in the AFS and the SAFS was higher than the biomass of the banana plants, even though the planting density of the associated trees was lower. Herbaceous biomass also differed between production systems (Figure 13A, Table 6). The MCS org had the highest herbaceous biomass production, contributing up to 21.6% of the total aboveground living biomass. On the

contrary, in the MCS conv herbaceous biomass was significantly reduced to only 2.1% of the total aboveground living biomass. In the AFS, herbaceous biomass was lower than in full-sun MCS, contributing <1% to the whole aboveground living biomass. No differences were found between the AFS conv and the AFS org. More herbaceous biomass was produced in the SAFS than in the AFS, with a share of 2.6% of the total aboveground living biomass.

4.4.4. Cocoa and system yield

The cocoa yield was higher in the MCS than in the AFS, and the SAFS had the lowest yield (Figure 13B, Table 6). Higher yields were obtained in the MCS conv compared to the MCS org, while no differences were found between the AFS org and AFS conv were found.

Banana bunches dominated the total system yield in the AFS conv and the AFS org (Figure 13B). Over the whole year, 564 bunches ha⁻¹ were harvested in AFS conv and 490 bunches ha⁻¹ in the AFS org, corresponding, respectively, to 16.8 Mg ha⁻¹ and 15.6 Mg ha⁻¹ of fresh weight of marketable fruit. Only 273 bunches of bananas were harvested in the SAFS, representing 6.7 Mg ha⁻¹ of fresh weight. However, the fruits of the peach palm (3.1 Mg ha⁻¹ of fresh weight) and the araza shrub (0.03 Mg ha⁻¹ of fresh weight), as well as the ginger (0.1 Mg ha⁻¹ of fresh weight) and turmeric (1.2 Mg ha⁻¹ of fresh weight) tubers, complemented the products harvested in the SAFS. Figure 13B shows the dry weight for comparison.

4.4.5. Soil nutrients

The soil had a pH of around 7, with no differences between the various production systems (Table 7). The nitrogen content was higher in the two organically managed systems than in their respective conv counterparts (Table 7), while the opposite tendency was observed in relation to the phosphorous content: lower in both the MCS org and the AFS org than in the MCS conv and the AFS conv. The content of K_{ex} was not affected by the type of production system, but there was a tendency to reach higher values in the org systems. Mg_{ex} was lower in the AFS than in the MCS; the lowest content of Mg_{ex} was measured in the AFS org. The K_{ex}:Mg_{ex} ratio was dominated by the presence of magnesium in the soil, but it increased from the MCS to the AFS and from AFS conv to AFS org and to SAFS.

4.5. Discussion

4.5.1. Cacao fine roots and cacao biomass

Lateral cacao roots spread horizontally around the stem. Nygren et al. (2013), for instance, described a cacao rooting system with a horizontal diameter of up to 4.8 m. Therefore, we expected to find cacao fine roots at all distances from each cacao tree to the next, but with decreasing abundance towards the middle between two neighboring stems. The fine root system is supposed to develop close to the coarse roots to enable carbohydrate transfer from coarse to fine roots (Nygren et al., 2013). We excavated an individual cacao coarse root and followed it up, and found that it reached the stem of the neighboring cacao tree at a 4m distance (data not shown). Similar observations of far reaching cacao roots have been reported (personal communication), but in the literature there is a lack of further information on the horizontal distribution of the cacao rooting systems. None of the cacao fine root parameters neither the biomass changed with distance from the stem. Similar results for tree root length and biomass according to distance from the tree row were observed by Livesley et al. (2000). Cacao trees under all growing conditions were able to develop a widespread and homogeneously distributed fine root system close to the surface over the whole plot area (Abou Rajab et al., 2016; Abou Rajab et al., 2018; Kummerow et al., 1982; Nygren et al., 2013). The 1.7 m distance from the stem measured in this study may not have been sufficient to detect differences in root development, should there be any. However, these differences will always be difficult to detect in a cacao plantation, given that trees are usually planted between 3 and 4 m apart. The expected increase of cacao root development due to the influence of fertilizer and compost application around the stem at approximately 1.2 m from the stem, as shown for coffee plantations (Defrenet et al., 2016), was not observed: the homogeneous horizontal distribution of cacao fine roots might imply a well distribution of nutrients, at least up to 1.7 m from the stem.

Also contrary to our expectations, the different growing conditions of the various cacao production systems, i.e. the different fertilization regimes, cover crops, associated trees, banana plants, additional crops, etc., did not influence the cacao fine root distribution parameters, indicating that cacao fine root growth was not constrained by any limiting factor, neither in MCS nor in AFS where cacao trees grow together with associated trees.

We observed an increase of the fine root:shoot ratio of the cacao trees from the MCS to the AFS to the SAFS that was also described for MCS and mixed systems by Borden et al. (2017). This increase of the fine root:shoot ratio was due to the decreasing AGB of cacao trees from the MCS to the AFS, while the cacao fine root biomass did not change. Most probably, light was the limiting factor for the aboveground cacao growth in the AFS (Niether et al., 2018), while the cacao fine root systems developed without limitations in the MCS, as compared to the AFS. The root:shoot ratio for other crops like maize decreases with N-fertilization (Anderson, 1988).

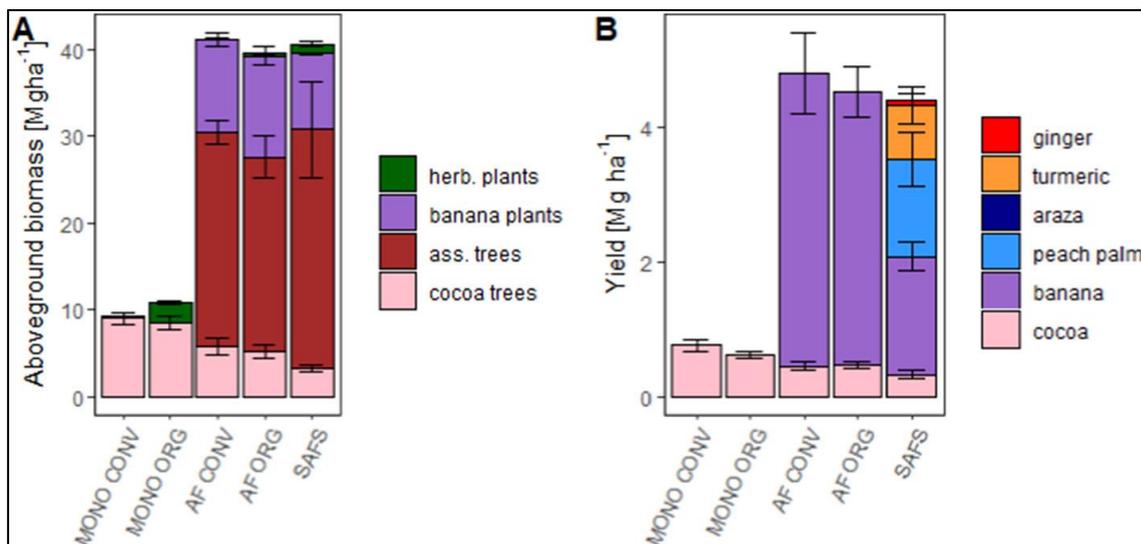


Figure 13: Aboveground production shown as (A) total standing biomass, and (B) total dry yield. In five cacao production systems.

Monoculture conventional (MONO CONV), monoculture organic (MONO ORG), agroforestry conventional (AF CONV), agroforestry organic (AF ORG) and successional agroforestry system (SAFS).

Neither the application of N-fertilizer in the conv plots nor the higher N content in the organically-managed plots compared to those conventionally-managed affected the fine root:shoot ratio. Only the increase of the cacao fine root biomass from the AFS conv to the AFS org to the SAFS implied a decrease in nutrient availability (Anderson, 1988), which might be explained by the use of compost in the AFS org, compared to the use of mineral fertilizer in the AFS conv and the lack of both compost and readily soluble mineral fertilizer in the SAFS. Our findings also contradict the results of Abou Rajab et al. (2018), who have described the same amount of cacao fine root biomass in cacao MCS and simple AFS, but only half of the amount of cacao roots in mixed cacao AFS with various tree species. Despite the lower biomass, they found a higher specific root length and specific root area in cacao fine roots grown in mixed systems than in those grown

in MCS (Abou Rajab et al., 2018), whereas these parameters did not change in our study. These differences between the different production systems, and between those results and ours, may be due to different soil conditions for cacao production, but also to increasing cacao tree density from MCS to AFS in their study (Abou Rajab et al., 2016; Abou Rajab et al., 2018), which may have made intraspecies interaction of cacao trees more likely. Species composition and soil conditions, as well as additional plantation characteristics such as tree management, age and fertilization regime, make comparison between sites difficult. All in all, the cacao fine root biomass in our results was only half the amount described in Abou Rajab et al. (2018) and Nygren et al. (2013), which may be explained by the lower cacao stem density and basal area in our trial (see Niether et al., 2018). Additionally, the cacao tree crowns in our trial were regularly pruned, which may not have been the case in on-farm trials. Crown pruning can also explain shifts in the cacao fine root production, and may lower the amount of living fine root stock in the soil by reallocating C to the shoot (Chesney and Nygren, 2002; Defrenet et al., 2016).

Table 6: Results from the linear mixed-effects model for standing aboveground biomass, herbal biomass, fine root:shoot-ratio and yield in five cacao production systems. results

Component of the system	Standing aboveground biomass				Yield			
	Cocoa trees	Banana plants	Associated trees	Herbaceous plants	Total	Cocoa trees	Banana	Total
Analysis of variance System	F value	31.1 ***	2.5	57.2 ***	87.9 ***	F value	13.6 ***	38.5 ***
	t value	10.3 ***	NA	6.4 ***	18.6 ***	t value	6.6 ***	-12.4 ***
Orthogonal contrast MONO vs AF	F value	8.1 ***	2.1 n.s.	-2.9 *	7.2 ***	F value	4.9 ***	-4.6 ***
	t value	0.9 n.s.	NA	-12.2 ***	2.5 *	t value	2.2 *	0.5 n.s.
MONO CONV vs MONO ORG								
AF CONV vs AF ORG								

MONO CONV: monoculture conventional; MONO ORG: monoculture organic; AF CONV: agroforestry conventional; AF ORG: agroforestry organic; SAFS: successional agroforestry system; F values: analysis of variance; t values: orthogonal contrasts; levels of significance: * p < 0.05; ** p < 0.01; *** p < 0.001; n.s. non-significant.

Table 7: Soil pH and soil nutrients in five cacao production systems and

	pH	N [%]	P [mg kg ⁻¹]	K _{ex} [cmolc kg ⁻¹]	Mg _{ex} [cmolc kg ⁻¹]	K _{ex} :Mg _{ex} -ratio
MONO CONV	7.7	0.15 ± 0.00	22.3 ± 1.5	0.43 ± 0.07	1.9 ± 0.2	0.25 ± 0.06
MONO ORG	7.4	0.19 ± 0.01	14.0 ± 0.0	0.54 ± 0.13	1.8 ± 0.2	0.31 ± 0.08
AF CONV	7.3	0.16 ± 0.02	29.0 ± 5.7	0.44 ± 0.04	1.7 ± 0.2	0.27 ± 0.04
AF ORG	7.2	0.21 ± 0.02	20.0 ± 2.2	0.63 ± 0.05	1.3 ± 0.1	0.53 ± 0.09
SAFS	7.7	0.18 ± 0.01	18.7 ± 3.9	0.62 ± 0.10	1.4 ± 0.1	0.47 ± 0.07
Analysis of variance System	F value	3.6	2.8	1.6	4.9	7.2
	t value	-1.1	-1.4	-1.1	-3.4	-3.6
Orthogonal contrast MONO vs AF	F value	0.5	0.8	0.8	2.0	3.6
	t value	-0.6	2.0	1.9	-0.3	-1.2
MONO CONV vs MONO ORG						
AF CONV vs AF ORG						

MONO CONV: monoculture conventional; MONO ORG: monoculture organic; AF CONV: agroforestry conventional; AF ORG: agroforestry organic; SAFS: successional agroforestry system; F values: analysis of variance; t values: orthogonal contrasts; levels of significance: * p < 0.05; ** p < 0.01; *** p < 0.001; n.s. non-significant.

4.5.2. Below- and aboveground system production

The lack of differences in the cacao fine root standing biomass between MCS and AFS allows us to affirm that differences in the total fine root production within the ingrowth donuts must be mainly due to the development of roots from other species.

In the MCS conv, only cacao trees were grown and herbaceous plants were kept at a minimum by spraying herbicides to reduce interspecies competition. Therefore, the total fine root production corresponded mainly to the cacao root growth. The cacao trees in the MCS conv, where interspecies specific root competition was kept at a minimum level, had the highest cacao bean production compared to the other production systems, as it was also shown for intensively managed full-sun cacao production systems in Ghana (Ahenkorah et al., 1974) and Asia (Vaast and Somarriba, 2014).

Both the MCS conv and the MCS org had the same light conditions, which enabled the cacao trees to develop a similar biomass. However, the organically-managed cacao trees produced less beans than their conventionally-managed counterparts, as it is often the case when comparing crop yields under org and conv management (Seufert et al., 2012). Nutrient availability is challenging in org agriculture due to the different sources of fertilization input, i.e. mineral fertilization with readily-available nutrients and compost application with organically-bound nutrients (de Ponti et al., 2012). The surplus of fine roots produced in the MCS org is mainly explained by the over crop, since the cover crop was spreading over the whole soil surface and thereby efficiently controlling the growth of other herbaceous plants. Although the leguminous cover crop was systematically removed from around the cacao stems, its roots were growing very close to them and developed strongly in the same soil layer as the cacao roots. The cover crop produced more fine roots than the cacao trees, especially in relation to its much lower AGB. This may have increased the belowground competition for nutrient uptake (Lehmann et al., 2000; Schroth et al., 2001) and consequently widened the yield gap between the MCS conv and the MCS org. Despite the higher nitrogen content in the soil of the organically-compared to conventionally-managed plots, the former showed a tendency to have less available phosphorous in the soil, which may result from a high uptake of this nutrient by the cover crop. Further competition for trace nutrients between cacao trees and

cover crop may have been the cause for the lower yield.

In the AFS, the surplus of fine root production, as compared to the situation in the MCS conv, was due to the roots of the associated trees and crops. Abou Rajab et al. (2018) have also shown an increase in fine root biomass from MCS to mixed cacao systems, but mainly below a 60 cm depth, while they did not report any differences between 0 and 40 cm of depth, in contrast to our findings. This might be explained by differences in tree species and soil characteristics between sites, but also by differences in the planting density of the cacao and associated trees. In both AFS and in the SAFS, the cacao tree biomass and bean production were lower than in full-sun systems. Radiation was strongly reduced by the shade canopies inducing light limitation (Niether et al., 2018), but also the higher production of fine roots could have retarded the development of the cacao trees by root competition for available macro- and trace nutrients. Both the limitation of light for photosynthesis and the different availability of nutrients for bean production explain the cocoa bean yield gap between MCS, AFS and the SAFS. Similarly, to the cacao biomass and yield, the herbaceous biomass was highly reduced in agroforestry

systems due to lower light levels and to the application of herbicides in the AFS conv, as shown by studies performed in the same site (Niether et al., 2018 and Schneider et al., 2017, respectively).

Interestingly, org and conv management did not affect the cocoa bean production of the AFS conv and the AFS org (Schneider et al., 2017), while the cocoa bean production and biomass of the SAFS were lower. This fact cannot be explained by higher root competition, but might be related to the higher total stem density in the SAFS, which came along with high shading and a different pruning history (Niether et al., 2018), and with the lack of any kind of fertilizers (Schneider et al., 2017). In contrast to the reduced aboveground production in the MCS org compared to the MCS conv, the cocoa yield reduction in the AFS was compensated by the high total biomass and production of other crops, especially bananas, which increased the economic return of the total system (Armengot et al., 2016). Furthermore, the high crop diversity of the SAFS offset the lower cocoa and banana yield with the production of fruit and tuber crops that might help improve and diversify family income or diet (Schroth et al., 2001). Additionally, the

total AGB and total fine root production were much higher in both AFS and in the SAFS than they were in the MCS, implying a high above- and belowground C storage potential (Abou Rajab et al., 2016; Schneidewind et al., 2018) and an improvement of the soil quality. This is shown by the increase of the Kex:Mgex ratio from the MCS to the AFS to the SAFS, which came close to the value of 0.7:1 that is supposed to be optimal for plant nutrition in clay soils (Loide, 2004) such as those in the present study (Niether et al., 2017a).

While aboveground stratification depends on the species characteristics and the type of management implemented by the farmer, the belowground organization of the roots depends as well on the soil structure and characteristics (Isaac et al., 2014), making information on belowground interaction even more difficult to obtain and transfer to other regions. The Lixisols and Luvisols of the Alto Beni region (Schneider et al., 2017) show the typical increase of clay with depth, accompanied with a very high and increasing bulk density (Niether et al., 2017a) that makes rooting more difficult (personal observation). Roots of associated trees in AFS are therefore more likely to develop closer to the soil surface and to the cacao rooting systems, and to compete for the same soil space and, therefore, for the same resources, such as nutrients and water. In all production systems, the majority of the total fine roots were produced in the upper soil stratum and only 25% of the cacao fine roots were grown in the 25–50 cm layer. These results are in line with other studies of cacao production systems (Abou Rajab et al., 2018; Kummerow et al., 1982). We observed a higher total fine root production in both soil depths of the AFS and the SAFS, compared to the MCS, indicating that roots of species other than cacao trees occupied these spaces. Even though the distance of the donut to the next woody tree was at least 3 m, according to the planting pattern, tree roots may spread far and occupy a large area. For instance, the *Inga edulis* tree, one of the species in our AFS, develops root systems with a horizontal diameter of >8 m and the highest fine root biomass located in the upper soil layer (Nygren et al., 2013).

The density of associated trees in the SAFS was higher than in the AFS, and herbaceous plants also contributed considerably to the high fine root production observed in that system. In addition, banana plants were placed in the AFS with the same initial planting density as the cacao trees, resulting in a mean distance from the cacao tree of 2.8 m. In

the SAFS, the banana planting density was lower than in the AFS, but the individuals were taller (Niether et al., 2018) and the AGB of the banana plants was the same. More than 65% of the roots of banana plants are also located in the first 0 to 30 cm of soil and can spread up to 5 m from the plant (reviewed by Blomme, 1999). Both associated trees and banana plants contributed to the total root biomass of the AFS and the SAFS, especially in the upper soil stratum where the highest share of cacao roots was located. But the roots of the associated species also occupied the space below the cacao roots (Nygren et al., 2013; Schwendenmann et al., 2010), as shown by the higher total root production in the lower soil stratum in the AFS and the SAFS, when compared to the MCS conv. This led to an increase in resource exploitation via occupation of a larger soil volume (Livesley et al., 2000) and to the building of a deep root safety net for capturing nutrients leached through the shallow cacao rooting zone (Nygren et al., 2013; van Noordwijk et al., 2002). Water absorption from the deeper soil layers by the associated trees, as compared to the cacao trees (Niether et al., 2017a), may have also enabled water uptake during dry periods (Schwendenmann et al., 2010). Deep-growing roots may further improve the quality of the heavy soil by increasing the soil org matter content in depth, via a higher infiltration rate and aeration of the soil (Schroth et al., 2001), and may also facilitate the presence of soil micro- and macrofauna deeper in the ground.

4.5.3. Leguminous cover crops and trees

The role of the leguminous cover crop must be further elucidated. Schneidewind et al. (2018) describe a higher nitrogen content in the cacao leaves and branches of the organically-managed systems of this trial, while the nitrogen content of the beans did not change between systems (Niether et al., 2017b). Additionally, the water resources in the cacao rooting zone were not affected by the leguminous cover crop, as established by a study comparing conventionally- and organically-managed MCS in the same site (Niether et al., 2017a). Our results showed a higher N content in the soil of the organically-managed plot. The source of the nitrogen could be attributed to the N-fixation of the cover crop, but also to the annual compost application. The benefits of the leguminous cover crop are therefore doubted, as is the practicability of an organically-managed cacao MCS. In contrast, the org management of the AFS did not

reduce, unlike the conv management, the yield and the tree biomass and is, therefore, a suitable alternative (see also Schneider et al., 2017). The leguminous cover crop was strongly reduced to avoid light limitation and, consequently, so was its potential effect on root competition with the cacao trees.

Despite the possible competitive effect, leguminous trees in AFS (species from the *Erythrina* and *Inga* genera, like those in this study) may also play a role in the nutrition of cacao trees. They may even support cacao fine root development and distribution by provision of nitrogen. This explanation may hold true for the enhanced cacao fine root performance due to the presence of the leguminous *Gliricidia* seen in AFS compared to cacao MCS by Abou Rajab et al. (2018).

4.6. Conclusions

Trade-offs between below- and aboveground competition and associated ecosystem services such as biomass production and yield diversification must be considered when discussing cacao AFS and MCS. We found spatial overlap of cacao roots and roots of the cover crop that may have increased competition for nutrients and led to yield reduction. Although the roots of associated trees in AFS may also compete with cacao roots, they increase the spatial exploration of the soil in depth and thereby encourage a higher total system yield and biomass. An optimized management of a cacao production system should combine maximization of the total system yield and minimal disruption of the ecosystem services. An org MCS with a cover crop such as the one managed in our trial is not an adequate solution, but org AFS can indeed compete with their conv counterparts.

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Contributions of authors

Wiebke Niether analyzed the data and wrote the first draft of the manuscript. Ulf Schneidewind and Michael Fuchs were responsible for the field experiments and data collection. Monika Schneider was the coordinator of the long-term trial in Bolivia, where the study was conducted, and established the field design. Laura Armengot was supervising this study. She supported in writing the manuscript and running the

statistical data analysis and interpretation of the results. All co-authored contributed to the manuscript by detailed reading and revision.

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5. Synthesis, conclusions and outlook

What still exists of "nature" is increasingly affected by humans. Closed forest stands are more and more fragmented or disappear entirely. Nature is being replaced by human landscapes, with the consequence of loss of biodiversity and vulnerability to extreme weather events. For economic reasons, forests are cleared to make way for intensively managed MCS. With the long-term consequences of a high input of agrochemicals. Alternative management practices to MCS, like AFS, as practiced in many societies for centuries, are considered traditional, inefficient and unprofitable.

In this study, the approach was chosen to compare the sustainability of different cacao production systems in terms of biomass accumulation and nutrient availability in order to assess the impact of land use and land cover changes from a landscape ecology perspective. To capture a large part of the C and nitrogen cycle of the production systems, both above-ground and below-ground pools were analysed. In addition, the return of above-ground biomass through litterfall and pruning was recorded, as well as the decomposition rates of litter and the microbial activity. The results were obtained using established standard methods from biology, forestry and environmental sciences. A substantial part of the results was collected through long-term field data collection, supplemented by laboratory analyses and statistically analysed on the computer. In the following, the most relevant findings of the three components of this thesis are presented with regard to the underlying question.

Concerning the results of the above-ground surveys (Chapter 2), they are very unambiguous (Figure 14). Total AGC stocks in the AFS significantly exceeded those in the MCS, although the biomass of cacao trees in the MCS was greater than in the AFS. However, after six years, the total aboveground biomass in the AFS is only one-third of the biomass in the tree population of the surrounding forests ($\sim 65 \text{ Mg C ha}^{-1}$; Yaffar, 2014). Nevertheless, an increase in biomass is to be expected, as total amounts of up to 50 Mg C ha^{-1} have been recorded in fully developed AFS in the same region (Jacobi et al., 2014).

The total amount of C circulating in the system through the annual pruning of both cacao and agroforestry trees is more than double the amount of litterfall in the corresponding system. The annual N input from pruning residues of cacao and especially agroforestry

trees can be up to ten times higher than the nitrogen input from external fertilisation if a large proportion of the agroforestry trees in the system are N-fixing leguminous.

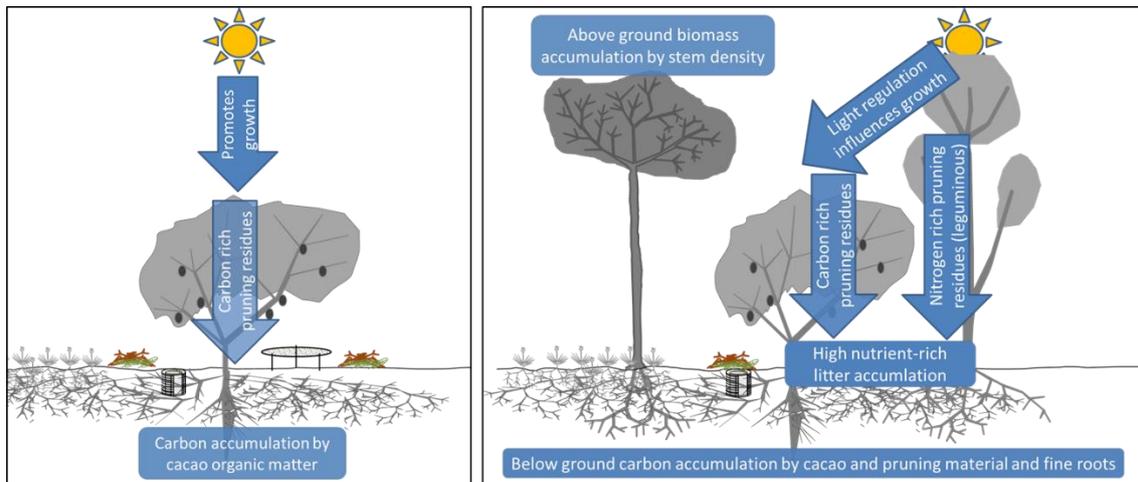


Figure 14: Synthesis of the comparison of monoculture systems (left) and agroforestry systems (right).

The findings on soil quality (Chapter 3) show that organically managed systems have accumulated more C and nitrogen in the topsoil under cacao trees after six years than conv cacao production systems of the same age. The greatest differences and the highest soil activity are in the topsoil, while in deeper soil horizons no or hardly any changes have taken place after six years. In terms of Cmic and Nmic, there were significant differences between the systems. The microbial nitrogen, for example, is up to four times higher in org managed systems, especially in org AFS. The experiment on the decomposition rates of leaf-litter material by organisms smaller than 1 mm showed no difference between the cultivation systems. However, the half-life of the decomposition of cacao leaves is twice as high as the decomposition rate of the nitrogen-containing *Erythrina* leaves.

As far as the biomass in the soil horizons (Chapter 4) is concerned, in particular the root mass until 50 cm soil depth, the main part of the cacao roots is found in the upper 25 cm. The horizontal cacao fine roots are homogenously distributed on the plot surface area. The roots of the other trees in the AFS also extend into the root zone of the cacao trees. In AFS and org MCS systems, root production within one year is up to 4 times higher than in conv systems.

This study shows that AFS accumulate more biomass in the system over the same period of time compared to MCS under the same initial conditions (soil, water and climate) (Figure 14). It was also possible to observe a chain of effects in AFS that also interacts with an organic management (Figure 15). The larger above-ground biomass makes it possible to stimulate a C and nitrogen cycle with targeted management through tree pruning. Depending on its chemical composition, the resulting litter is either completely decomposed by microorganisms and is available to plants again, or is incorporated into the soil compartment in the form of stable C. Whichever form prevails, by recycling the biomass in the form of litter, the health and fertility of the soils is preserved to the greatest possible extent. AFS thus offer the possibility of working without external input and with org standards. Thereby, the composition of trees in an AFS is crucial for a balance between nutrient-rich and C rich litter (lignin). On lean sites with little nutrients and low cation exchange capacity, it is therefore recommended to rely on fast-growing plants with high nitrogen content during the implementation phase. For sites with better soil conditions, the proportion of fast-growing trees that remain in the system for a shorter time, can be kept at a lower level. Organically managed cacao MCS are indeed also an option in terms of avoiding the use of agrochemicals. However, there are still unanswered questions regarding long-term and a sustainable management.

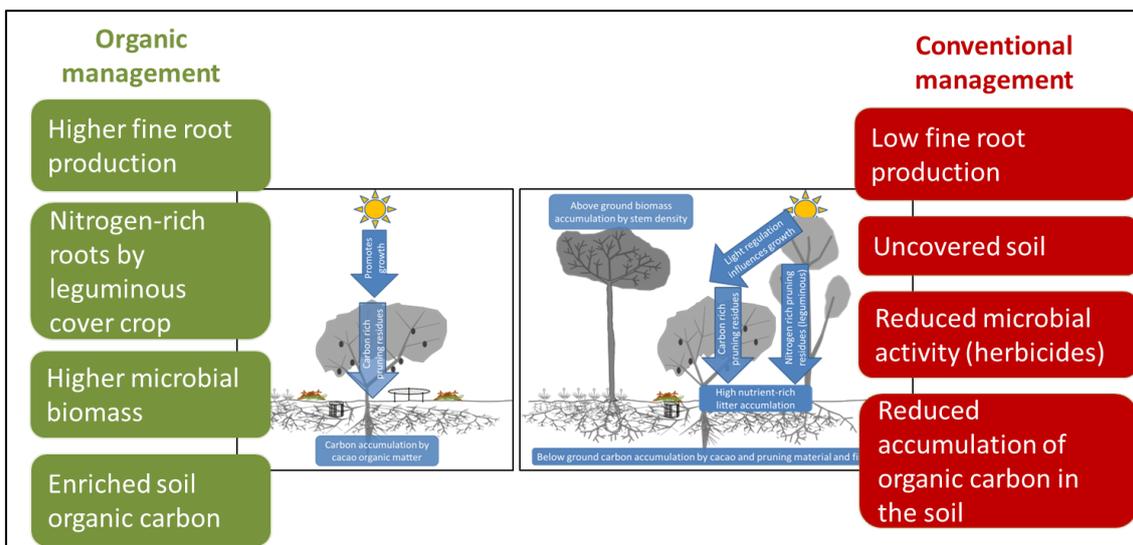


Figure 15: Synthesis of the comparison of organic managed systems (left) and conventional managed systems (right).

Since agricultural land is always a production system and therefore a source of income and livelihood provision, maximizing the overall yield of the system should be combined

with minimal degradation of ecosystem services. Nevertheless, smallholder agroforestry is not designed for short-term profit, but for the preservation of long-term ecosystem services and sustainable production.

Overall, this study intends to show how the type and intensity of agricultural use affects the landscape. Deforestation and changes in land use practices cause direct alterations in the landscape. These changes towards agricultural land almost inevitably lead to a reduction of the ecosystem quality. This also applies to AFS, for which species-rich primary and secondary forests are being cleared. Nevertheless, AFS are preferable to MCS, with regard to the ecological properties, and promoting the expansion of well-managed AFS is recommended.



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