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Alternative Scenarios for the Development of Oil Palm in the Tropics: Carbon and Nutrients Dynamics

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Dedication

To my beloved daughter: Elsa Dariela Quezada Aplicano.

Mi amada hija tan lejos y tan cerca a la vez, eres el mejor regalo de la vida.

Ma fille bien-aimée, si lointaine et si proche à la fois, tu es le plus beau cadeau de la vie.

*“**Utopia** is on the **horizon**.*
*I move two steps closer; it **moves** two steps further **away**.*
I walk another ten steps and the horizon runs ten steps further away.
*As much as I may **walk**, I'll never **reach** it.*
So what's the point of utopia?
*The point is this**: to keep walking**”*

Eduardo Galeano

II Summary

Expansion of agriculture in forested areas poses significant threats and pressure to ecosystems and potentially the global climate. More specifically, expansion of oil palm (OP) plantations has been found to exert great impact on key ecosystem functions like C storage, water cycling and support biodiversity. Alternative pathways for a more sustainable development of agriculture have been proposed. However, lack of evidence through comprehensive studies constrains the potential of deforestation free options to be accounted as a fundamental part or as priority scenarios of future sustainable OP development. This thesis work looks to provide insights into the effects of two alternatives land use change options into OP: planted pastures and savanna conversion on soil biogeochemical aspects and ecosystem C storage.

To investigate such impacts, a space-for-time substitution or chronosequence approach was used. All the field sites of this thesis work were located in the eastern savanna region of Los Llanos in Colombia. OP plots were of known age and were selected from three representative long-established commercial OP farms to be part of two chronosequences. For one chronosequence, OP plots were located in the Piedmont region of Los Llanos, close to the Andean mountains, and was made of OP plots established on former pasture areas that included first and second cycle plantations of: 12-, 18-, 30-, 32-, 36-, 45- and 56-year-old (a long-term chronosequence). The other chronosequence of OP plots was located in the well-drained high plains savannas area of Los Llanos (Altillanura), at approximately 200 km apart from the other chronosequence. This chronosequence of OP was established on former natural savanna and OP plots were of: 2-, 4-, 8-, 9-, 12-, 23- and 27-year-old, thus encompassing only the first cycle of plantation.

A common tool used across all the studies of this work was the natural abundance of C stable isotopes. In the case of pastures and savanna conversion into OP, a shift of C_4 into C_3 vegetation takes place and therefore it was possible to elucidate valuable aspects of soil C dynamics like the fate of old C4, savanna or pasture, and new OP-derived C, as a consequence of land use change (LUC). Another important aspect of this work, is that soils were sampled down to 50- and 70-cm, something which is rather rare in the literature. With this deep sampling strategy, I was able to report changes that took place in subsoil layers. Finally, a highly relevant feature of this thesis work is the assessment of management practices in parallel with LUC effects. To do so, I sampled soils according to the so-called management zones in OP plantations.

Management zones are well-defined areas with contrasted management practices (four in total in mature plantations) that are imposed over long time periods. For example, application of most fertilizers along the OP cultivation cycle is made in a circular area around the palm trunk named weeded circle. For all the previously noted characteristics, it is clear that the conversion of pastures or savannas into OP plantations can be considered as a long-term natural ^{13}C labeled experiment combined with

different management practices and thus an ideal model to assess important C cycling aspects like stabilization, decomposition and C turnover in bulk soil and also in SOC fractions under field conditions.

Pastures conversion into OP was C neutral at the ecosystem level (Manuscript 1), however soil C storage decreased by 39% during the first OP cycle, ca. 30 years, and then remained stable along the second OP cycle. This ecosystem C neutrality contrasts remarkably with the deforestation scenario for OP development, where about 173 Mg C ha^{-1} are lost due to the establishment of OP plantations. Another positive implication of establishing OP in pasture areas was the increased chemical fertility. However, this trade-off between soil C storage and chemical fertility demands close attention so that soil fertility could be enhanced mainly by means of soil natural processes rather than by adding chemical inputs. Such reliability on SOC to enhance soil fertility in OP plantations is possible in the long-term as indicated by the found recovery of labile C fractions and bulk soil C content in the topsoil (Manuscript 4).

In the conversion of savannas into OP plantations, a rather positive ecosystem C balance was found (Manuscript 2). Such C gain at the ecosystem level was mainly explained by the no change in soil C stocks over an entire OP rotation cycle, which in turn was due to the slow decomposition of savanna-derived C and effective OP-derived C accumulation that offset the small savanna C losses. Importantly, soil biological activity and SOC stocks were found to be maintained or even enhanced, when organic amendments (i.e. crop residues from frond piles) were used. This contrasted, with the practice that relied heavily on mineral fertilizers application to increase chemical fertility. It was also demonstrated that changes in SOC stocks within plantations are mainly driven by C inputs that are controlled by OP fine roots (Manuscript 3). The fine OP roots were directly affected by nutrients addition and drive changes in soil microbial properties.

This work demonstrates that alternatives LUC to OP can reduce significantly the C losses associated with deforestation. Future OP development should be directed toward degraded pastures areas so that the initial SOC losses are not as high as found in the well-managed pastures of this work. Notably, the management decisions will play a key role on the direction and strength of SOC stock changes and can further increase the positive ecosystem C outcomes and soil C storage capacity of OP plantations in former savanna or pasture areas. Therefore, OP cultivation should shift toward a more ecologically oriented intensification with a better balance between environmental and productivity goals.

III Résumé

L'expansion de l'agriculture dans les zones forestières fait peser des menaces et pressions importantes sur les écosystèmes et, potentiellement, sur le climat mondial. En particulier, il a été constaté que l'expansion des plantations de palmiers à huile (OP) a un impact considérable sur les fonctions clés des écosystèmes comme le stockage du carbone (C), le cycle de l'eau et la biodiversité. Des voies alternatives pour un développement plus durable de l'agriculture sans déforestation ont été proposées. Cependant, le manque d'études approfondies de ces alternatives limite le potentiel de ces options à être considérées comme une partie fondamentale ou comme des scénarios prioritaires du développement durable futur des OP. Ce travail de thèse vise à montrer les effets de deux options de changement d'utilisation des terres pour les plantations de palmeraies à huile : les effets de la convertion des pâturages et celle des savanes sur les propriétés biogéochimiques des sols et le stockage du carbone dans l'écosystème.

Pour étudier ces impacts, une approche substituant le temps par l'espace en étudiant des chronoséquences a été utilisée. Tous les sites de terrain de ce travail de thèse étaient situés dans la région des savanes orientales de Los Llanos, en Colombie. Les parcelles d'OP étaient d'âge connu et ont été sélectionnées dans trois fermes commerciales représentatives pour la région. Pour la première chronoséquence, les parcelles d'OP étaient situées dans la région piémontaise de Los Llanos, près des montagnes andines, et étaient toutes établies sur d'anciennes zones de pâturage, en incluant des plantations du premier et du deuxième cycle de rotation de la culture, soit de 12, 18, 30, 32, 36, 45 et 56 ans. L'autre chronoséquence était située dans la zone des savanes des hautes plaines bien drainées de Los Llanos (Altillanura), à environ 200 km de la première chronoséquence. Cette chronoséquence a été établie sur d'anciennes savanes naturelles et les ages des parcelles d'OP étaient de 2, 4, 8, 9, 12, 23 et 27 ans, n'incluant ainsi que le premier cycle de plantation.

Une approche commune utilisée dans toutes les études présentées dans ce travail a été celle de l'abondance naturelle des isotopes stables du C (signature 13C), afin d'identifier l'origine de carbone organique du sol (SOC). Dans les deux cas de convertion des pâturages et des savanes en OP, un changement de végétation de type C4 en C3 a lieu. Ceci permet d'élucider des aspects fondamentaux de la dynamique du C du sol lors du changement d'utilisation des terres (LUC), comme le devenir de l'ancien carbone C4 de la savane ou du pâturage, et l'accumulation du nouveau carbone C3 dérivé des palmiers. Un autre aspect important de ce travail est que les sols ont été échantillonnés jusqu'à 50 et 70 cm de profondeur, ce qui est plutôt rare dans les travaux publiés. Grâce à cette stratégie d'échantillonnage en profondeur, j'ai pu détecter les changements qui se sont produits dans les couches profondes du sol. Enfin, un aspect très important de ce travail de thèse est l'évaluation des pratiques de gestion dans les plantations, en parallèle avec les effets du LUC. Pour ce faire, j'ai échantillonné les sols selon les zones dites de gestion au sein des plantations. Les zones de gestion sont des zones bien définies et contrastées (quatre au total dans les plantations matures) qui sont marquées sur de longues périodes. Par exemple,

l'application des engrais se fait essentiellement dans une zone circulaire autour du tronc des palmiers, appelée « weeded circle ».

Ensemble, ces caractéristiques font que notre étude de la conversion des pâturages et savanes en plantations OP peut être considérée comme une expérience naturelle sur le long terme de marquage au 13C en lien avec les pratiques de gestion. C’est donc un modèle idéal pour évaluer en conditions réelles de terrain des aspects importants du cycle du C, comme la stabilisation, la décomposition et le renouvellement du C dans le sol, soit globalement, soit dans les fractions du SOC.

La conversion des pâturages en OP a montré un bilan neutre en termes de C au niveau de l'écosystème en entier (manuscrit 1), mais le stockage du C dans le sol a diminué de 39 % pendant le premier cycle de plantation, soit jusqu’à environ 30 ans, et est resté stable pendant le deuxième cycle de la plantation. Cette neutralité en C au niveau de l'écosystème contraste remarquablement avec le scénario classique de la déforestation pour le développement des plantations d'OP, où environ 173 Mg C ha^{-1} sont perdus suite au LUC. Une autre implication positive de l'établissement des plantations d’OP dans les zones de pâturage a été l'augmentation de la fertilité chimique. Toutefois, ce compromis entre le stockage du carbone dans le sol et la fertilité chimique exige une attention particulière, de manière à ce que la fertilité du sol puisse être améliorée principalement par le biais de processus naturels dans le sol en lien avec le SOC, plutôt que par l'ajout d'intrants chimiques. Une telle perspective pour améliorer la fertilité des sols dans les plantations d’OP est possible à long terme, comme l'indique la récupération des fractions de C labiles et la teneur en C du sol dans l’horizon de surface (manuscrit 4).

Lors de la conversion des savanes en OP, un bilan positif de C au niveau de l'écosystème en entier a été démontré (manuscrit 2). Ce gain de C au niveau de l'écosystème s'explique principalement par le fait que les stocks de C du sol n'ont pas changé pendant la durée du premier cycle de rotation de la plantation, ce qui est dû à la lente décomposition du C dérivé de la savane et à l'accumulation efficace de C dérivé des OP qui compense les faibles pertes de C de la savane initiale. Il est important de noter que l'activité biologique du sol et les stocks de SOC se sont maintenus, voire ont augmenté avec les amendements organiques provenant des résidus de culture (décomposition des litières de feuilles). Cette situation contraste avec la pratique qui repose uniquement sur l'application d'engrais minéraux pour augmenter la fertilité chimique. Il a également été démontré que les variations des stocks de SOC au sein des plantations sont principalement dues aux intrants de C en lien avec les racines fines d'OP (Manuscrit 3). Les racines fines sont directement affectées par l'ajout de nutriments et entraînent des changements dans les propriétés microbiennes du sol.

Ce travail démontre que les LUC alternatifs pour les plantations d’OP peuvent réduire de manière significative les pertes de C associées à la pratique de la déforestation. Le développement futur des OP devrait être orienté vers les zones de pâturages dégradés afin que les pertes initiales de SOC ne soient pas aussi élevées que celles constatées dans les pâturages utilisés dans ce travail, qui correspondaient à des pâturages non dégradés avec un sol relativement riche en carbone. En particulier, les décisions de gestion joueront un rôle clé pour la direction et l’intensité des changements de stocks de SOC. Avec une gestion judicieuse, il est possible d’améliorer encore le bilan positif en matière de C de l'écosystème et la capacité de stockage du C du sol des plantations faites dans les anciennes zones de savanes ou de pâturages. Par conséquent, l’intensification des cultures d’OP devrait évoluer vers une pratique plus écologique, avec un meilleur équilibre entre les objectifs environnementaux et ceux de la productivité.

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1 General introduction

“Labor omnia vincit”

Field work in Colombia. On the picture at the bottom, from left to right: Andres Etter, Alexandre Buttler, Juan Carlos Quezada, Thomas Guillaume and Johanna Ruegg

1.1 Expansion of oil palm plantations in the tropics

Over the last decades, production and consumption of palm oil (PO) has increased worldwide at accelerated rates. This unprecedented increase in PO production has attracted substantial public attention due to the observed trade-offs between economic opportunities and environmental costs. PO has a number of advantages over other vegetable oils that range from human health benefits (i.e. replacement of *trans*-fatty acids) to multiple applications in the food and non-food industry and agronomic performance (most productive oilseed crop and long-life span). Despite all these advantages and others, the expansion of this agricultural commodity in the tropics has been heavily criticized by environmentalists and consumer groups. Southeast Asia is the main PO producing area worldwide by far: Indonesia and Malaysia produce the vast majority of world's PO supply (~85%) on nearly 10 million hectares (FAO, 2013). In these countries, a sizeable area of oil palm (OP) expansion occurred in newly deforested areas (Koh and Wilcove, 2008; Vijay et al, 2016). Albeit, West Africa is the center of origin of OP (Barcelos et al., 2015), productivity is extremely low in this region of the world compared to industrial scale plantations and other OP producer countries (Hoyle and Levang, 2012; Nkongho et al 2014). In fact, in countries like Nigeria and Cameroon between 70 to 80% of the land devoted to OP cultivation is under smallholder tenancy. Some authors have suggested that closing the yield gap (difference between actual yields and attainable yield potential), which is particularly large among smallholders, to increase OP fruit bunches yields holds big potential, particularly in West Africa (Woittiez et al, 2017).

Key concerns surrounding the OP debate include, but are not limited to, its detrimental environmental impacts that arise from land use change (LUC) in the tropics. LUC adversely impacts a number of ecosystem functions and services including regulation of climate, soil stabilization, carbon (C) sequestration and biodiversity (Koh et al., 2011; Garcia-Ulloa et al, 2012). Recent estimates showed that deforestation rates in Indonesia (0.84 Mha yr^{-1}) have surpassed those in Brazil, estimated at 0.43 Mha yr^{-1} (Margono et al., 2014). A significant share of this high deforestation rate (nearly 50%) provided the new land open for OP agriculture in Indonesia and Malaysia during the period between 1989-2013 (Vijay et al, 2016). The fast expansion of OP on forested areas poses high risks of changes in C ecosystem balance. Indeed, Henders et al. (2015) estimated that large-scale clearing of forested areas in Southeast Asia resulted in emissions of roughly 0.3 Gt of CO_2 yr^{-1} (second largest source after beef production due to LUC in the period 2000-2011). The expansion of OP on peatlands is of special concern in Indonesia and Malaysia given the substantial amounts of C that can be lost from both the above and belowground components of these ecosystems (Koh et al., 2011).

1.2 Alternatives to deforestation for oil palm expansion

Tropical land-use (LU) systems vary greatly in their ecosystem C storage. Values from 350 Mg C ha^{-1} in primary undisturbed forest systems to 52 Mg C ha^{-1} in grasslands/annual cropping systems can be found (Hairiah et al., 2001). In tropical forests the largest pool of C is in the aboveground biomass and they contain more C per unit area than any other land cover (Gibbs et al., 2007). Given these immense amounts of C stored in tropical forests, different kinds of anthropogenic perturbations such as LUC are key factors contributing to the release of terrestrial C stocks into the atmosphere. In fact, there is a general consensus that conversion of forest LU to OP agriculture leads to high C losses, being the use of peat soils in forests the one with the highest impact (net C loss of ~156 Mg of C ha^{-1}) on terrestrial C balances across tropical soils (Henders et al., 2015, Koh et al., 2011). However, perennial OP plantations store more C than other agricultural land uses (i.e. annual crops) (Sayer et al, 2012).

If LUC is to be less impactful on ecosystem C storage, land covers of low or moderate aboveground C (i.e. grasslands and cropland) have to be targeted for the expansion of OP agriculture. It has been estimated that to consider an OP plantation C debt free it has to replace LU systems that contain aboveground C stocks of less than ~42 Mg of C ha^{-1} (Khasanah et al., 2015). This replacement of less C-rich LU systems is the case of Latin America’s OP expansion, where previously cropped and grazed land (largest pool of C in soils and not in vegetation) are the main sources of new land for this industry (Furumo and Aide, 2017; Garcia-Ulloa et al., 2012).

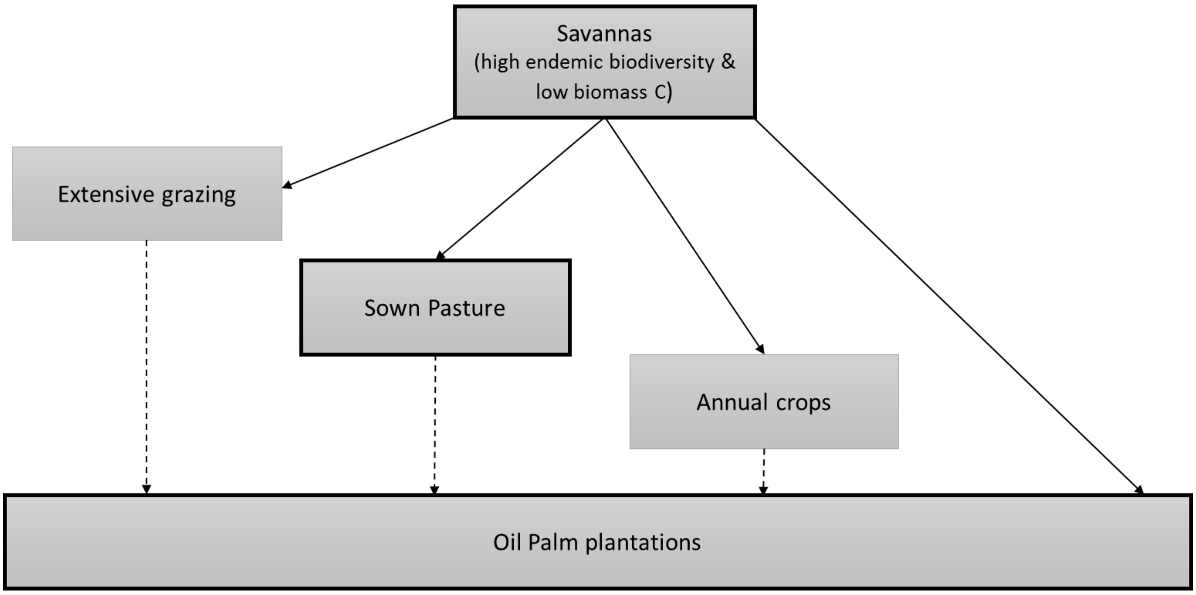


Figure 1. Conceptual model on the expansion of oil palm agriculture in Los Llanos region of Colombia (adapted from CIAT, 2004). Continuous lines indicate direct conversion, whereas dotted lines indicate conversion to oil palm from previous land uses. The bottom box indicates some peculiarities of OP systems in Colombia that are explained afterward in this report. Frames with ticker outlines are the deforestation free LUC types addressed in this thesis. The expansion of this commodity at the expense of less biodiverse and C-poor agroecosystems could set the stage for a novel more sustainable development for the future expansion of highly profitable

crops in the tropics. In Latin America, OP plantations grew by almost 46,000 ha y^{-1} (7% per annum) between 2000 and 2014, with land used for agricultural purposes (i.e. pastures, bananas) being the major source for new OP fields (60 to 70%) (Vijay et al, 2016; FAO, 2017; Furumo and Aide, 2017). As suggested by Garcia-Ulloa et al. (2012), OP expansion into lands with low C stocks is perceived as less harmful to the environment. In Colombia, the leading PO producer in Latin America, the source of new land for OP expansion also includes the use of semi-natural isohyperthermic savannas (Amezquita et al, 2002) located on the eastern side of this South American country (Fig. 1). Colombian savannas, so-called Los Llanos region, is an area experiencing extreme pressure due to the expansion of the agricultural frontier and OP is one of the fastest spreading crops (Lavelle et al., 2014). In 2014, Los Llanos region accounted for about 42 and 37% of the land devoted to OP cultivation and total PO produced, respectively (Fedepalma, 2015). Los Llanos region covers a broad extent (17 million ha). It presents a suite of biophysical conditions that favor the development of the OP sector (Romero-Ruiz et al, 2010). Moreover, government policies and incentives programs to promote biodiesel production and consumption have encouraged the OP expansion in the region as well (Castiblanco et al., 2013).

According to Castiblanco et al. (2013), the development of the OP sector in Colombia is mainly occurring at the expense of pasture lands (51%), although a higher pasture conversion to OP (58%) is reported in Los Llanos. Forecasted scenarios predict a rapid increase of the OP industry in this region (Castiblanco et al., 2013). While factors like flat topography, light well-structured soils and good drainage have underpinned the expansion of the agricultural frontier in Los Llanos, there are also some soil and climate related conditions that challenge the optimal development of agricultural activities in this area. Most soils in this region are highly weathered Oxisols and Ultisols, which are characterized by having low P content (~2 ppm), low effective cation exchange capacity (ECEC) (< 5 meq 100 g^{-1}) and organic matter (2.5%), high aluminum saturation (86%) and acidity (<5) (CIAT, 2004; Basamba et al., 2006). Given these savannas soil characteristics and the constant removal of nutrients in OP fruits, there is consequently a demand of high inputs in the form of fertilizers to sustain high OP yields.

1.3 Soil C and nutrient dynamics in the savanna region of Colombia

The ongoing LUC in Los Llanos to OP agriculture may greatly influence soil organic C (SOC) dynamics. Extensive cattle ranching on either native savanna species or introduced pasture species is the main land use system in Los Llanos (CIAT, 2004). For example, Fisher et al. (1994) found that replacement of native savanna vegetation by deep-rooted introduced grasses like *Andropogon gayanus* and *Brachiaria humidicola* increased soil C sequestration by up to 70 Mg C ha^{-1}. In contrast, OP plantations have a shallow root systems with more than 80% of the total root biomass distributed in the upper 60 cm (Cuesta et al, 1997; Jourdan and Rey, 1997). Nevertheless, there are no studies on the dynamics of C in OP plantations following pasture systems cultivated with either introduced grasses or native savanna grass species in Los Llanos region.

The shift from C_4 (pasture and savanna species) to C_3 (OP) vegetation cover opens up an opportunity to assess SOC changes by measuring the relative abundance of the stable isotopes ^{13}C and ^{12}C. It is common knowledge that plants performing the C_3 photosynthetic pathway discriminate more against ^{13}C than those under the C_4 mechanism. This results in distinct ^{13}C signatures in soils that can be large enough if soils are predominantly covered either by C_4 ($\delta^{13}C$ value ~-12‰) or C_3 vegetation types (i.e. OP $\delta^{13}C$ value of -29‰) (Lamade et al., 2009) for long periods. In the case of the savannas of Los Llanos, the vegetation is dominated by C_4 tropical grasses (mainly *Andropogon* and *Trachypogon* grass species). In the case of pastures, the dominant species is the introduced *Brachiaria spp*. However, with the ongoing expansion of OP, the old C_4 carbon of savannas and pastures is being replaced by the new C_3 OP-derived carbon. Hence, by examining changes over time (chronosequence approach) in C isotopes ratios ($\delta^{13}C$) of C_4 soils converted to OP, we can potentially obtain valuable *in situ* estimations of SOC turnover. To date, few studies (Goodrick et al., 2014) have made use of stable isotopes analysis to evaluate C dynamics in C_4 soils, mainly because only a small amount of C_4 soils are used for OP cultivation globally.

In addition to the importance of SOC on global C balance, soil productivity and quality is greatly determined by the reservoir of C in soils (van Noordwijk et al., 1997). A review assessing the production-ecological sustainability of nine major feedstock crops for biofuel production revealed that, in terms of soil quality and other indicators, OP performed better than many other crops (de Vries et al., 2010). However, current knowledge on the effects of OP cultivation on long-term soil fertility, after more than two OP rotation cycles (>50 years), is scarce due to the lack of documented field evidence. Commercial operations of OP plantations in Los Llanos began about 50 year ago (Rueda and Pacheco, 2015), which allows investigating the long-term dynamics of soils. To date most studies have focused on the impacts of mature or old first-cycle OP plantations on soil indicators such as fertility, SOC and biological activity (Law et al., 2009; Smith et al., 2012; Frazao et al., 2013; Lee-Cruz et al., 2013; Goodrick et al., 2014; Frazao et al., 2014; McGuire et al., 2014; Allen et al., 2015; Guillaume et al., 2015; Kotowska et al., 2015; Goodrick et al., 2016;). Moreover, none of the existing reports has addressed the long-term effects of OP cultivation on soil sustainability in time series. Thus, a scientific knowledge gap exists concerning how the soil changes in response to long-term OP cultivation. It is crucial to examine the changes in soil quality and long-term trends of C storage of land under OP cultivation beyond one OP cultivation cycle for assessing the sustainability of OP systems. This long term, sustainability is also important for potential future new LU after OP.

1.4 Management effects of OP plantations on C and nutrient dynamics

Besides LUC, SOC dynamics also depends on crop management practices (Lal, 2005). In fact, the use of management practices that can increase SOC stocks and support long-term soils productivity are being promoted (i.e. zero-tillage and manure use) in agricultural systems. Typically, OP plantation management includes a number of crop management practices (i.e. accumulation of pruned fronds within the plantation and assignation of exclusive areas for mechanizations of activities), that creates high spatial variability in this agroecosystem. In recent years, research have found highly variable C and N stocks between OP management zones (MZ) (Frazao et al., 2014; Frazao et al., 2013; Law et al., 2009). This strong spatial heterogeneity within plantations has to be considered in order to make a proper characterization of the properties of each of OP-specific MZ (Nelson et al., 2015). There are four OP-specific MZ: interzone (IZ); harvest path (H), weeded circle (W) and frond pile (F) (Fig. 1 in Manuscript 3). The gradual formation of 2, 3 and ultimately 4 MZ is a consequence of palms growth, development and the incorporation of crop management practices that seek to facilitate operations like transportation and collections of fruit bunches and mechanization of field operations. In general, an OP plantation is started with palms of about twelve to fourteen months' year-old that were grown in a nursery.

Management practices in the IZ zone are minimal throughout the plantation cycle. This area covers the majority of the ground surface area in OP operation, ranging from ~88 to 60% in a young (2 years-old), mature and old plantation (>5 years-old), respectively. With the implementation of new management practices, this area is now being seeded with perennial legume cover crops, which are considered as a mean of improving some soil physical, chemical and biological indicators. Along with the IZ zone, the W zone is delineated since the beginning of the field operations. The proportion of the W zone relative to the total plantation area varies from ~12% in a young plantation to 15% in an old one (>15 years-old). On these circular zones surrounding the trunk of the palm's fertilizers are spread until 3-4 years after transplanting and they are generally kept free of any vegetation over all the plantation lifespan. Lastly, the two remaining zones (H and F) appear after the third or fourth year of OP establishment and cover about 50% of the ground in a mature plantation. The H zone serves as an avenue for mobilization of field machinery that carry out various crop operations (i.e. fruit transportation, mechanization of fertilizers applications), whereas F is the site for accumulation of pruned fronds. In spite of the significant impacts that MZ can have on soil and OP aspects, few studies have focused on the characterization of effects of MZ. Since MZ are kept constant during a given cultivation cycle and operations are maintained over long time periods, they serve as a research model worthy of investigation and that will very likely result in significantly different outcomes. Although not yet widely adopted, OP agronomic management practices could encompass the use of advanced crop management practices like the use of leguminous cover crops. For example, Kudzu (*Pueraria phaseoloides*) is a shade tolerant tropical legume (Congdon and Addison, 2003) well adapted to low fertility acid savanna soils (Sylvester-Bradley et al., 1988). Such legume-OP association is thought to be a means to improve OP agronomic performance and profitability, while reducing the negative impacts of using external chemical inputs such as herbicides and fertilizers. Moreover, including cover crops in the management of agricultural soils contributes to increase soil C accumulation (Dinesh et al., 2006). The use of symbiotic N-fixing plant species in annual cropping systems has been successfully implemented and is well documented. However, the use of legumes intercropped with oil palm plantations is not yet well understood.

1.5 Thesis overview

Soils are currently of global interest due to their capacity to be a solution to cope with some of the foremost challenges for humanity that include climate change and food security. A growing area of interest in soils research lies in their potential to sequester atmospheric carbon and with that mitigate the risks of global warming. Furthermore, increasing carbon storage in soils will also help to improve soil quality on the long term, which in turn will increase global food production and the environmental sustainability of agriculture. For these previously noted soil aspects, this thesis aims to assess the impacts of deforestation-free alternatives on soil biogeochemical properties and ecosystem C storage for a more sustainable expansion of OP agriculture in the tropics. As presented in figure 1, this work focuses on the conversion of natural savannas and pastures into OP. In general, we hypothesized that both savanna and pasture conversion into OP will positively impact ecosystem C storage. However, SOC storage and nutrient dynamics will follow different patterns in pastures and savanna derived OP plantations due to the distinct initial SOC stock levels and the distinct characteristics of old C_4-C (i.e. mineralogy and SOC pools). Also, soils under different MZ will exhibit different biogeochemical properties according to the type of inputs applied (i.e. organic amendments and synthetic fertilizers). Each of these general hypotheses and others along with well-defined objectives, results and conclusions are presented in the following chapters of this thesis. In the last part of this section, table 1, a brief summary of the manuscripts prepared for submission to peer-reviewed journals or already published are presented.

Manuscript 1 (Chapter 2): Carbon neutral expansion of oil palm plantations in the Neotropics
Aim: assess the pasture-to-OP transition as an alternative to deforestation to mitigate the net ecosystem C losses of future OP expansion.

Manuscript 2 (Chapter 3): Deforestation free expansion of oil palm and improved management to reduce C footprint
Aim: assess the effect of savanna conversion to OP, as a deforestation free alternative, on ecosystem C storage and to explore how management practices could improve the C footprint of this LUC type.

Manuscript 3 (Chapter 4): Drivers of soil carbon stabilization in oil palm plantations

Aim: disentangle the effects of soil C and nutrient inputs on newly accumulated SOC (OP-derived) and the decomposition of old SOC (savanna-derived), taking advantage of the specific patterns of fertilization and soil C inputs in the four MZ.

Manuscript 4 (Chapter 5): Long-term changes in soil organic carbon fractions and natural ^{13}C abundance following pastures conversion into oil palm plantations

Aim: bridge some knowledge gaps in the assessment of OP expansion on SOC dynamics. More specifically we sought to: 1) determine which SOC fractions are the most affected by pasture conversion into OP, 2) determine the ^{13}C isotopic composition of C pools and 3) quantify fundamental parameters of SOC fractions dynamics like stabilization and decomposition rates, time necessary to reach steady state of stocks and mean residence time (MRT).

1.5.1 Study sites

All the field sites of this thesis work were located in the eastern savanna region of Los Llanos in Colombia. Plots of known age within three representative long-established commercial OP farms were carefully selected to be part of two chronosequences. The primary criterion for selecting plots was land use history so that the selected OP plantations had a single preceding LU (sown pasture or natural savanna). Plots with steep slopes, inundated parts or located on floodplains and with distinct management to the standard farm practices were excluded.

For manuscripts two and five, OP plots were located in the Piedmont region of Los Llanos, close to the Andean mountains, at the large scale OP farm of La Cabana. It is worthy to note that this large-scale operation (roughly 3,000 ha) could have the longest history of OP cultivation in all Los Llanos region. The established chronosequence was made of OP plots that included first and second cycle plantations of: 12-, 18-, 30-, 32-, 36-, 45- and 56-years after pasture conversion. Reference sites for these OP plots were three adjacent cattle ranching farms (total study area of roughly 5,000 ha). Soils in this chronosequence were inceptisols of about 60-70 cm depth overlaying alluvial sediments. Piedmont soils have greater fertility than those in the well-drained savannas.

For manuscripts three and four, a chronosequence of OP plots located in the well-drained high plains savannas area of Los Llanos (Altillanura), at two commercial farms (Sapuga and Sillatava), were included. This chronosequence had four reference sites that were natural savanna areas surrounding the selected OP plantations. Ages of OP plantations were: 2-, 4-, 8-, 9-, 12-, 23- and 27-year-old (only first cultivation cycle). The use of one or other chronosequence in the four different manuscripts of this thesis was defined according to the study scope and objectives of each piece of research work as briefly detailed in table 1.

Table 1. Brief summary of manuscripts prepared during this thesis work

Mansucript	**Research focus**	**Study area**	**Some key features**	**Journal**	**Submission status**
1	Pastures conversion into OP	Piedmont region of Los Llanos (Cabaña farm)	✓ Long-term (two rotation cycles) C4-C3 approach ✓ Subsoil effects due to LUC	Science Advances	Published Nov-2019
2	Savanna conversion into OP	Altillanura region of Los Llanos (Sapuga farm)	✓ Combined analysis of LUC and management effects on soil biogeochemical properties across one full rotation cycle ✓ Brief synthesis of deforestation free LUC options of this thesis on ecosystem C storage	Nature Sustainability	Under revision since April 2020
3	Savanna conversion into OP	Altillanura region of Los Llanos (Sillatava farm)	✓ Soil biogeochemical aspects in MZ of a mature plantation ✓ Mechanisms underlying new OP-derived C accumulation	Land Degradation and Development	Published June-2019
4	Pastures conversion into OP	Piedmont region of Los Llanos (Cabaña farm)	✓ Long-term effects of LUC on SOC fractions and natural ^{13}C abundance	Soil Biology and Biogeochemistry	Under preparation

2 Manuscript 1: Carbon neutral expansion of oil palm plantations in the Neotropics

*“**Discipline** will sooner or later **defeat intelligence**”*

Field work in the plantations in the pastures region of Los Llanos (Cabaña farm and adjacent cattle ranching farms

Juan Carlos Quezada, Andres Etter, Jaboury Ghazoul, Alexandre Buttler, Thomas Guillaume

Status: Published in Science Advances, November 2019

2.1 Abstract

Alternatives to ecologically devastating deforestation land use change trajectories are needed to reduce the carbon footprint of oil palm (OP) plantations in the tropics. Although various land use change options have been proposed, so far, there are no empirical data on their long-term ecosystem carbon pools effects. Our results demonstrate that pasture-to-OP conversion in savanna regions does not change ecosystem carbon storage, after 56 years in Colombia. Compared to rainforest conversion, this alternative land use change reduces net ecosystem carbon losses by 99.7 ± 9.6%. Soil organic carbon (SOC) decreased until 36 years after conversion, due to a fast decomposition of pasture-derived carbon, counterbalancing the carbon gains in OP biomass. The recovery of topsoil carbon content, suggests that SOC stocks might partly recover during a third plantation cycle. Hence, greater OP sustainability can be achieved if its expansion is oriented toward pasture land.

2.2 Introduction

Rainforests have been a major source of land for newly established oil palm (OP) plantations, particularly in the main OP-producing countries such as Indonesia and Malaysia (Gaveau et al. 2016; Vijay et al. 2016). Conversion of rainforests to OP plantations negatively impacts a number of ecosystem functions including ecosystem carbon (C) storage, soil fertility and biodiversity (Dislich et al. 2016; Fitzherbert et al. 2008). Indeed, in Southeast Asia, a global hotspot of greenhouse gas emission from deforestation and land use change, deforestation for OP cultivation was the second largest source of CO_2 emission (~0.3 Gt of CO_2 yr^{-1}) (Henders, Persson, and Kastner 2015). Replacement of forested areas by OP plantations reduces ecosystem C storage by up to 173 Mg C ha^{-1} owing, mainly, to the abrupt loss of biomass (Boucher 2011; Guillaume et al. 2018). Such ecosystem C losses are exacerbated when OP plantations are established on tropical peatlands, as has occurred in Southeast Asia (Koh et al. 2011; Lucey et al. 2014).

In response to the detrimental environmental impacts associated with deforestation, various deforestation-free land use change trajectories have been proposed for a more sustainable OP expansion, including the use of marginal lands and conversion of savannas and pasture areas (Garcia-Ulloa et al. 2012; Koh and Ghazoul 2010). Of these, the use of pasture areas has great interest given the vast amount of land under pastoral systems, and their low biodiversity and biomass C stocks (Fitzherbert et al. 2008; Gilroy et al. 2015; Ziegler et al. 2012). This land use change even has potential for climate change mitigation benefits by increasing C sequestration, given the substantially higher aboveground biomass C of OP over pastures (Garcia-Ulloa et al. 2012). Yet, large uncertainties remain (Furumo and Aide 2017; Vijay et al. 2016) particularly with respect to changes in total ecosystem C (TEC) changes, SOC stocks and other soil properties. Meta-analysis studies on the conversion of pastures into perennial plantations (often called afforestation) showed contrasting SOC stocks changes (Guo and Gifford 2002; Li, Niu, and Luo 2012). Only two recent studies have quantified the effects of grasslands and pastures conversion into OP plantations on SOC storage, but their results are in disagreement (Frazão et al. 2013; Goodrick, P. N. Nelson, et al. 2015). Studies are much more numerous in the typical deforestation land use change trajectory of OP development on forested land. Most report that forest-to-OP conversion leads to soil degradation and C losses due to decreased organic matter (OM) inputs and erosion (Dislich et al. 2016; Frazão et al. 2013; Guillaume, Damris, and Kuzyakov 2015; van Straaten et al. 2015),although a few have noted positive or unclear effects on SOC (Frazão et al. 2013; Ni'matul Khasanah et al. 2015; Smith et al. 2012). In addition and in spite of the generally accepted view on SOC losses in OP plantations and the major role of soil organic matter (SOM) on soil productivity, soil chemical fertility and plant nutrient availability appear not to be negatively impacted by OP agriculture, likely due to the use of mineral fertilizers in plantations (Allen et al. 2015; Dislich et al. 2016).

The reported changes in SOC following land use change to OP have occurred mainly in the surface layers (0-30 cm) and no effects in the subsoil have been detected. Yet the focus of the literature to date has been the first OP rotation cycle (25-30 years) or even shorter time periods which might not be a sufficient time to realize the effects of ongoing belowground processes. Evidence from other land use change types have already demonstrated that time after vegetation change (Bárcena et al. 2014; Guo and Gifford 2002; Paul et al. 2002) and sampling depth (Don, Schumacher, and Freibauer 2011) have major roles on the magnitude and direction of changes in SOC stocks. The time following land use change needed for soils to reach either a new C equilibrium state or a subsequent recovery after the initial C losses usually occurs only after several decades (Don et al. 2011; Paul et al. 2002). Indeed, in a recent comprehensive review on ecosystem functions in OP, the need to consider longer-term studies to assess the effects of OP cultivation on ecosystem functions was emphasized (Dislich et al. 2016). The lack of observed responses in subsoil layers might be explained by the short time periods considered after land use conversion. Inputs of fresh OM from deep palm roots and nutrients from leaching might occur and stimulate the microbial mineralization of the large SOC stocks stored in subsoils (Fierer, N., Allen, A., Schimel, J. & Holden 2003; Fontaine et al. 2007). The absence of change in SOC stocks in subsoils does not necessarily indicate that the old SOC stabilized during the previous land uses was not affected by the conversion, as it might instead be substituted by new SOC derived from OP inputs. This can be investigated when OP, which has a C3 photosynthetic pathway, is established on tropical pastures or savannas, which are dominated by grasses having a C4 photosynthetic pathway (Medina 1982). The different fractionation intensities of the two photosynthetic pathways result in differences in the isotopic signature ($\delta^{13}C$) of the biomass, enabling the origin of SOC to be determined (Balesdent, Mariotti, and Guillet 1987). This provides a powerful tool to study decomposition and stabilization of SOC after land use change.

In Colombia the expansion of OP plantations has occurred mainly on pastures planted on cleared savannas and, to some extent also on native tropical savannas. These land use changes contrast with the deforestation trajectories of the main OP producing countries in southeast Asia (Castiblanco, Etter, and Aide 2013; Furumo and Aide 2017). Oil palm coverage in Colombia, which is currently the fourth major OP producer worldwide, has increased rapidly, tripling in the last two decades from roughly 160,000 ha to 480,000 ha (Rueda-Zárate and Pacheco 2015). Of this expansion, about 60% involved the use of low productivity pasture areas (Castiblanco et al. 2013). Nearly half of the land devoted to OP cultivation in Colombia occurs in the Llanos region of eastern Colombia, where cattle ranching is the main land use (Fedepalma 2015; Guimarães et al. 2004), and future scenarios predict a fast growth rate of the OP industry in this region (Castiblanco et al. 2013).

This study aims to assess the pasture-to-OP transition as an alternative to deforestation to mitigate the net TEC losses of future OP expansion. Specifically, we hypothesized that C gains in OP biomass might be counterbalanced by C losses in the soil. SOC stocks would be affected down to 50-cm but a new equilibrium would be reached in all layers after two rotation cycles. Because of the long-term chemical fertilization in OP plantations, we expect to observe tradeoffs among soil ecosystem services such as C storage and nutrient provision. To test these hypotheses, we quantified the dynamics of OP biomass C stocks, OP- and pasture-derived SOC stocks down to 50-cm and nutrient availability over a long-term 56-years chronosequence of OP plantations established on former pastures in Colombia, taking advantage of a shift from C4 to C3 vegetation.

2.3 Material and methods

2.3.1 Study area

The study was carried out in “La Cabaña”, a large-scale commercial OP plantation (73°22'W, 4° 16'N) and three adjacent cattle ranching farms, close to the town of Cumaral, of Meta Department in Colombia. The area is located in the piedmont of the Llanos region in the eastern plains, close to the Andean mountains, with an altitude of 300 m (Figure S1). The climate in the area is tropical with a well-marked dry season that last from December through March. Annual rainfall is of about 3,400 mm and annual mean temperature is approximately 27°C. The study area is located on the well-drained Pleistocene and late Tertiary alluvial terraces, where soils are predominantly dystrophic Inceptisols (Oxic Dystropepts) of about 60-70 cm effective depth overlaying coarse alluvial sediments, on flat topography.

In general, the Llanos is a vast territory mostly dominated by a mosaic of savanna (C4-dominated) and gallery forest ecosystems that over the past few decades has undergone fast land use change to intensive commercial agriculture and is often regarded as one of the last frontiers for agricultural expansion in South America (Guimarães et al. 2004; Health and Complete 2004). The region accounts for about 1/4 of the national territory, respectively 22 million ha (Guimarães et al. 2004; Romero-Ruiz et al. 2010). Cattle ranching is the predominant land use in the piedmont and of the Llanos in general, where large areas of cleared forests and savannas have been sown with improved varieties of Brachiaria grasses for several decades (Etter, Sarmiento, and Romero 2010) (*Brachiaria* spp.). *Brachiaria* grasses are of African origin and they are widely used in improved South-American C4-pasture systems. However, during the last 40 years, increasing areas of these pastures and natural savannas have transitioned to intensive agriculture of rice and OP plantations. Due to the economic and social benefits, a suit of favorable climatic conditions in the Andean piedmont of the Llanos and increasingly governmental stimulus, OP plantations are being established since more than half a century and their expansion is predicted to continue (Rueda-Zárate and Pacheco 2015). As is the case for most OP plantations in Colombia, the study site plantations at La Cabaña were derived from pastures, which had been planted on former well-drained grasslands savannas.

2.3.2 Approach and study sites

We used a space for time substitution approach (chronosequence) to quantify the long-term impacts on biomass and soil properties, including changes in SOC stocks and soil chemical fertility characteristics, following pastures conversion into OP plantations. As we aimed to study the long-term effects of OP cultivation on soil properties, OP plots with palms stands passing by the first rotation cycle (up to 30 years) and the second rotation cycle (new palms transplanted after the first cycle) were considered. We selected six OP plots that range from 12 to 56 years after pasture conversion and three reference pasture sites. All OP plots were part of a large-scale OP plantation. This large-scale OP farm could have the longest history of OP cultivation in the whole Llanos region and its management is representative of the typical management of OP plantations in this region of Colombia. The reference sites were cattle ranching farms that were adjacent to the sampled OP farm. The primary criterion for selecting OP plots was the existence of pastures as the sole preceding land-use. This information was obtained by direct communication with the personnel in charge of both the OP farm operation and the neighboring cattle ranching farms. Additionally, in the plot selection process, sites with steep slopes, inundated parts or located on floodplains and with distinct management to the general farm practices were excluded. OP plots had been planted in a triangular design 9-m apart, which resulted in a density of 143 palms ha^{-1}. All OP plots, except the recently replanted 32-year plot, presented four well distinct management zones: i) the frond piles located in between palms and where pruned OP fronds are accumulated; ii) the harvest path which is the area of traffic for mechanized operation, i.e. fruit harvesting, in between parallel palm lines; iii) weeded circle which is an area around the palm trunk of about 5-m diameter where most fertilizer inputs are placed up until 5-6 years; and iv) the interrow where almost no field operations takes place and scattered understory vegetation, i.e. weeds, grows. Land was prepared prior plantation establishment by chisel plow to a depth of 10- to 15-cm. Dolomitic lime was added to increase soil pH. Fertilization practices were the typically recommended ones for the region and were done periodically every year in two applications on the weeded circle at young ages or broadcasted all over the plantation except the harvest path in mature plantations (over 5-year plantations). Nitrogen (N), phosphorus (P) and potassium (K) fertilization of 150 to 725 kg ha^{-1} yr^{-1} are applied depending on palm stand age, i.e. 725 kg NPK-complete fertilizer applied to plantation aging more than 10-year. Other nutrients including boron and magnesium were also applied regularly every year.

2.3.3 Sites selection and sampling

Soil samples were taken from a chronosequence of OP with stand ages of 12-, 18-, 30-, 32-, 45- and 56-year, the three first blocks corresponding to first cycle plantations and the last three to second cycle plantations. OP blocks were of sizes between 20-30 ha. In addition, three adjacent cattle ranching farms were sampled as reference sites. All selected sites were located within an area that covered roughly 5,000 ha.

Soil samples from OP plots were collected using a modified transect methodology (Nelson et al. 2015). This sampling strategy allows to have mixed samples that represent well the spatial variability in OP plantations due to management practices. Furthermore, it is also well adapted for measures in replanted plantations, since the spatial distribution of management zones differs between OP cycles. Twenty sampling points evenly spaced were marked along a 50-m diagonal transect at ca. >60°

(considering a selected palm at row 1 of the transect as reference point). The diagonal transects crossed six OP rows and the four management zones typically found in commercial OP plantations. To capture the spatial variability at each of the OP blocks, three parallel transects were made, except in the 12- and 32-year OP blocks, and sampled in 2016, with some complementary sampling in 2017. One transect was established in a centered position relative to the number of palms rows and average number of palms per row in each block. The other two transects were established at least 120 m away from each side of the first transect. Transects were established at least 50 m away from the plot's edges. At the three *Brachiaria* pasture sites, one diagonal transect of 50 m long oriented east-west with 20 evenly spaced sampling points was made. At all diagonal transects (OP blocks and pastures sites), a total of 20 soil cores were taken. Half of those cores were taken to a depth of 30 cm and the other half went down to 50 cm in an alternative manner, using a 6-cm diameter soil auger. Cores were divided into four depth intervals: 0-10, 10-20, 20-30 and 30-50 cm. Therefore, samples of the 0-10, 10-20 and 20-30 cm were composed of 20 cores and the 30-50 cm samples were made of 10 cores. Final samples in each transect were bulked into a composite sample resulting in one sample for each depth in each transect. Samples were homogenized, air dried, sieved through 2-mm, sealed in bags and stored at room temperature until transportation to the laboratory in Lausanne (Switzerland). Soil physical, chemical and isotopic analyses were carried out after drying at 35°C in forced-air ovens.

Pits were dug in a middle representative position along the first 50-m linear transects to determine soil bulk density (BD) in each OP block and one reference site to 70 cm depth. Two stainless steel volume cores were inserted horizontally into a pit wall at each depth increment. Soils from the two cores were mixed and oven dried at 105°C for 48 hours to calculate bulk density.

2.3.4 Above and belowground biomass

At the previously described central diagonal transects of each OP sampled plot, the heights of ten randomly chosen palms were measured. Palm height was measured from the palm base to the base of the youngest fully expanded leaf (Kotowska et al. 2015). Estimation of aboveground biomass was based on palm height using the allometric equation for mineral soils(Khasanah, van Noordwijk, and Ningsih 2015).

$$\text{AGB OP} = 0.0923(\text{height}) + 0.1333 \qquad (1)$$

Belowground OP biomass was estimated according to the allometric model based on OP plantation age (Göttingen and Syahrinudin 2005) (Eqn 2).

$$\text{BGB OP} = 1.45\ (\text{age}) + 9.88\ (143\ \text{palms ha}^{-1}) \qquad (2)$$

Biomass C stocks were estimated using a factor of 41,3% of above and belowground OP biomass (Göttingen and Syahrinudin 2005). Time-averaged C in OP biomass was estimated as the C stocks accumulated in the middle of one rotation cycle, thus the biomass C stock in a 30-year old OP plantation was divided by two (Penman et al. 2003).

Laboratory analyses

Soil particle size analysis was done on air-dried soils by the hydrometer method after removal of organic fraction with 30% H_2O_2 (G. W. Gee 1986). Soil pH was determined in a 1:2.5 soil to water slurry. Soil samples were extracted with Mehlich-III solution (Mehlich 1984) and analyzed for available cations including Ca, K, Na and Mg using an Inductively Coupled Plasma (ICP) spectrometer (PerkinElmer, Waltham, Massachusetts, USA). Exchangeable acidity was determined by extracting 2 g of soil with 10 ml 1N KCL, shaking for 30 min at 200 RPM. Samples were allowed to settle for 30 minutes, filtered and extraction funnels were washed three times with 30 ml 1N KCl. Titration was conducted with 0.01 N NaOH after adding phenolphthalein to the extract (Major et al. 2012). Effective cation exchange capacity (ECEC) was estimated by summing the amount of charge per unit soil (meq $100g^{-1}$) from major cations (Ca, K, Na and Mg) plus exchangeable acidity. Base saturation (BS) was obtained by dividing the total sum of charge per unit soil from Ca, K, Na and Mg by ECEC.

Total C and N contents, 13C and 15N were measured on air-dried and ground soil weighted in tin cups with an Elemental Analyser (Eurovector) coupled to an isotope ratio mass spectrometer (Delta plus, Thermo Fisher) at the stable isotope lab at the Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland. Total C content correspond to total organic content because of the absence of carbonates in heavily-weathered soils.

The SOC stocks were calculated as the product of soil bulk density, layer thickness and SOC concentration. The contribution of new and old C to total SOC was calculated as shown in Eqn. 3 (Balesdent and Mariotti 1996):

$$\mathrm{f} = (\delta\mathrm{sam} - \delta\mathrm{ref})/(\delta\mathrm{op} - \delta\mathrm{ref}) \qquad (3)$$

Where f is the relative proportion of OP-derived C (C3) in SOC stocks. δsam is the $\delta^{13}C$ of the soil sample and δref is the $\delta^{13}C$ of the corresponding soil depth from pasture as C4 reference soil. δop is the $\delta^{13}C$ of nine fine roots biomass C samples. The method accounts for the natural increase of $\delta^{13}C$ signature with depth generally observed in soils and neglects the ^{13}C fractionation that could occur at the first step of SOC formation.

2.3.5 Statistical analyses

Data analyses were performed using RStudio version 3.4.0 (R Development Core Team 2017) statistical software. Linear regression analysis was used to examine changes in soil chemical and physical properties (i.e. major cations, BD) relative to increase time after pastures conversion into OP plantations. Assumption of normality was checked for all analyses with Shapiro-Wilk test and visual inspection of normality plots. If assumption of normality was not satisfied, permutation tests were performed as in the case of Ca, sum of cations and BS. Patterns in changes of soil C stocks over the OP chronosequence time were examined for the bulk soil and for each of the four sampled soil layers using regression models. The nonlinear least square ‘nls’ and linear model ‘lm’ functions in R were used to fit non-linear and linear regression models, respectively. In addition, the ‘segmented’ function was used to perform segmented (broken-line) regression analysis. Statistical significance was declared at $p < 0.05$. Similarly, model fit for changes in C3-OP derived C and C4-pasture derived C over the chronosequence time, were also examined for each of the four studied soil layers by testing the above-mentioned models. These regression analyses allowed estimating rates of C3 accumulation and C4 decomposition and decay constants (*k*), rates of total soil C decrease and breakpoints in soil C stock changes (time at which a change in the direction of change in C stocks occurred).

After testing various models (i.e. mono-exponential, bi-exponential), model performance assessment was based on Akaike Information Criterion (AIC) and coefficient of determination (R^2) values. Models with the largest R^2 and lowest AIC values were selected (see Table S1). Accordingly, (i) changes in whole soil C stocks and C stocks in each soil layer were described by fitting segmented regression models (except linear regression on the deepest soil layer 30-50 cm), which yielded estimated breakpoints; ii) C3-derived C in the 10-20 cm, 20-30 and 30-50 cm soil layers were described by linear models, while pattern in C3-derived C in the soil surface layer (0-10 cm) was described by an exponential rise to equilibrium model of the form:

$$y = ((k * y0 - A) * \exp(-k * t + A)/k \tag{4}$$

where y is the C stock, k is the annual decay constant of the pool, y0 is the C3-C stock before OP cultivation started (thus 0), A is the C3 annual input to the C3 pool and t is time after conversion; iii) A first order decay model was fitted to the obtained pasture-derived C data in the four studied soil layers:

$$y = c*\exp(-k * t) \tag{5}$$

where y is the C stock, c is the original C stock before OP cultivation, k is the decay rate constant and t referred to time. The half-life (HL) of the original C stock in eqn. 5 and of the OP input in eqn. 4 was calculated as:

$$HL = \ln(2)/k \tag{6}$$

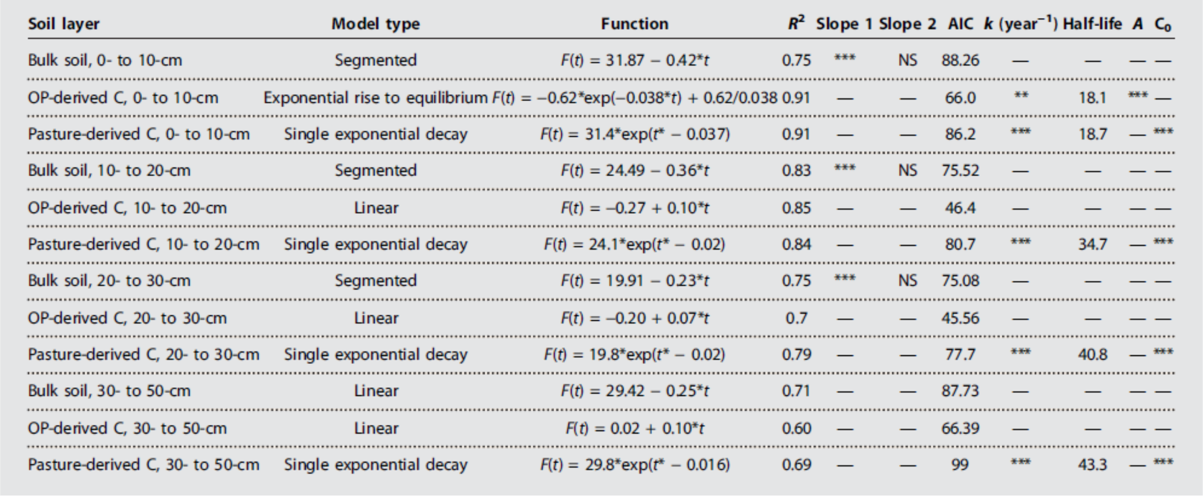
A principal component analysis (PCA) was carried out for further exploration of the relations between soil parameters that can be impacted by cultivation (BD, C:N, ^{13}C, C, N, EA, Na, pH, K, Mg, ^{15}N, Ca, BS and P).

2.4 Results

2.4.1 Soil C stocks dynamics

Cultivation of OP on former pasture areas severely affected SOC stocks to a depth of 50 cm (Fig. 1). Out of the 102 ± 8 Mg C ha^{-1} stored in pasture SOC, 39 ± 8 % were lost after 56 years of OP cultivation. However, the C loss dynamics followed neither a linear nor an exponential trend, but rather two trends in one adjusting to a segmented regression model (Fig.1, Table S1). SOC stocks down to 50 cm constantly decreased until the beginning of the second OP cycle (breakpoint: 36.1 ± 9.0 years) at a rate of 1.26 ± 0.26 Mg C ha^{-1} yr^{-1}, after which SOC stocks stabilized along the second OP rotation cycle.

Table 1. List of functions and kinetic parameters. Parameters describe changes in bulk SOC stocks and C3-derived and C4-derived C stocks in the four sampled soil layers. R2, coefficient of determination; slopes 1 and 2, significance of the two slopes for segmented regression analysis; K, rate constant; A, annual input of C3-C; C0, SOC stocks before pasture change to OP. **P < 0.01; ***P < 0.001.



Soil layer	Model type	Function	R^2	Slope 1	Slope 2	AIC	k (year^{-1})	Half-life	A	C_0
Bulk soil, 0- to 10-cm	Segmented	$F(t) = 31.87 - 0.42*t$	0.75	***	NS	88.26	—	—	—	—
OP-derived C, 0- to 10-cm	Exponential rise to equilibrium	$F(t) = -0.62*\exp(-0.038*t) + 0.62/0.038$	0.91	—	—	66.0	**	18.1	***	—
Pasture-derived C, 0- to 10-cm	Single exponential decay	$F(t) = 31.4*\exp(t* - 0.037)$	0.91	—	—	86.2	***	18.7	—	***
Bulk soil, 10- to 20-cm	Segmented	$F(t) = 24.49 - 0.36*t$	0.83	***	NS	75.52	—	—	—	—
OP-derived C, 10- to 20-cm	Linear	$F(t) = -0.27 + 0.10*t$	0.85	—	—	46.4	—	—	—	—
Pasture-derived C, 10- to 20-cm	Single exponential decay	$F(t) = 24.1*\exp(t* - 0.02)$	0.84	—	—	80.7	***	34.7	—	***
Bulk soil, 20- to 30-cm	Segmented	$F(t) = 19.91 - 0.23*t$	0.75	***	NS	75.08	—	—	—	—
OP-derived C, 20- to 30-cm	Linear	$F(t) = -0.20 + 0.07*t$	0.7	—	—	45.56	—	—	—	—
Pasture-derived C, 20- to 30-cm	Single exponential decay	$F(t) = 19.8*\exp(t* - 0.02)$	0.79	—	—	77.7	***	40.8	—	***
Bulk soil, 30- to 50-cm	Linear	$F(t) = 29.42 - 0.25*t$	0.71	—	—	87.73	—	—	—	—
OP-derived C, 30- to 50-cm	Linear	$F(t) = 0.02 + 0.10*t$	0.60	—	—	66.39	—	—	—	—
Pasture-derived C, 30- to 50-cm	Single exponential decay	$F(t) = 29.8*\exp(t* - 0.016)$	0.69	—	—	99	***	43.3	—	***

The dynamics of the total SOC stocks down to 50 cm resulted from the combination of variable rates and patterns in the accumulation of OP-derived and decomposition of pasture-derived C at different depths (Fig. 2). In the surface soil layer (0-10 cm), bulk SOC stocks decreased sharply at a rate of 0.42 ± 0.08 Mg C ha^{-1} yr^{-1} until 39.1 ± 4.5 years and then stabilized for the rest of the cultivation time. The initial decline in bulk SOC stocks was driven by a dramatic loss of pasture-derived C (77% after 39 years) that was not fully compensated by the accumulation of OP-derived C during the same period of time (Fig. 2A). The decomposition of pasture derived SOC followed a first order decay with a half-life time of 18.7 years (k = 0.037 ± 0.0038; Table 1). The accumulation of OP-derived SOC in the surface soil layer was best fitted by an exponential rise to equilibrium model, indicating a saturation in the accumulation of OP-derived C. The estimated gross OP-derived C input was of 0.62 ± 0.10 Mg C ha^{-1} yr^{-1} and its annual decay rate was of 0.038 ± 0.010 yr^{-1}. The decay rate constants of SOM between OP-derived and pasture-derived C were similar, suggesting no preferential C source for decomposers.

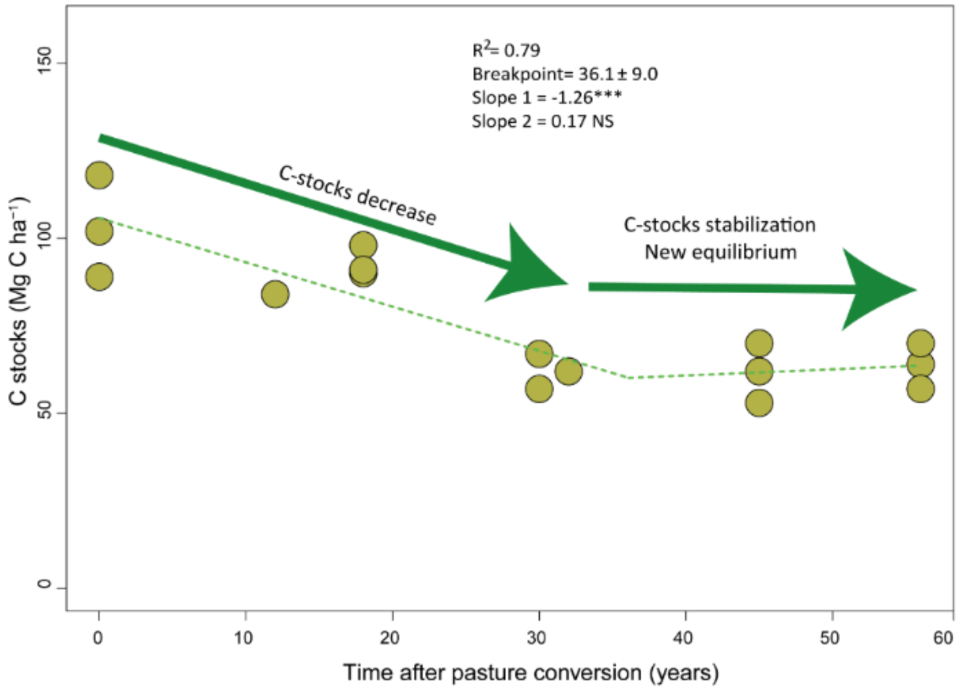


Figure 1. Soil carbon stocks after pasture conversion into OP plantations at 0- to 50-cm depth. The dashed line represents the fitted segmented regression equation. Significance of the slope coefficients from each side of the breaking point is indicated (***$p < 0.001$).

At breakpoint (39 years) the net difference between the kinetic rates of change (according to the difference between the first derivative of equations 4 and 5) was very low (-0.13 Mg C ha^{-1} yr^{-1}). SOC stocks in the top 10 cm would reach equilibrium at 16.33 Mg ha^{-1} (calculated as division between A and k parameters of equation 4), according to the model based on C isotopes, i.e. similar level as the one estimated by the segmented regression based on bulk SOC (15.46 Mg C ha^{-1}; table 1). These latter two estimations cross-validated the two approaches (bulk SOC and isotopic) used to estimate C dynamic in this work.

The SOC bulk stocks dynamics in the two deeper soil layers (10- to 20-cm and 20- to 30-cm) exhibited similar patterns, i.e. a constant decrease followed by a stabilization of the stocks along the second rotation (Fig. 2, B and C, and Table 1). However, the C loss rates for these two soil layers were 14% and 46% less pronounced than for the surface soil layer, but stabilized at similar time: 37.8 ± 4.4 and 38.9 ± 10.3 years for the 10-20 cm and 20-30 cm soil depths, respectively. The decomposition of pasture-derived SOC also followed an exponential decay in the 10-20 and 20-30 cm layers. The decay rates, however, were lower resulting in longer half-life of these pools as compared to the surface layer (18.7, 34.7 and 40.8 years for the 0-10, 10-20 and 20-30 cm soil depths, respectively). In contrast to surface soil layer, the accumulation of OP-derived C did not show any saturation, with constant accumulation rates of 0.10 ± 0.01 and 0.07 ± 0.01 Mg C ha^{-1} yr^{-1} for the 10-20 and 20-30 cm layers, respectively, throughout the two rotation cycles (Fig. 2, B to D; Table 1).

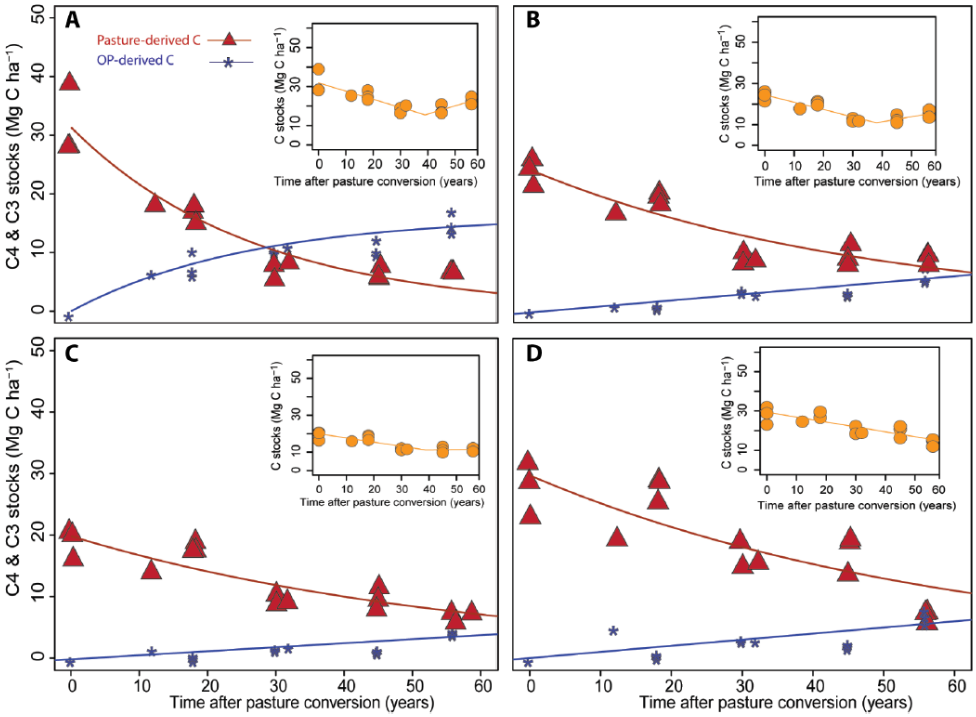


Figure 2. Dynamics of pasture C4-derived C and OP C3-derived C following pastures conversion into OP plantations. (A) 0- to 10-cm soil layer, (B) 10- to 20-cm soil layer, (C) 20-30 cm soil layer and (D) 30- t0 50-cm soil layer. Inset graphs show bulk SOC stocks for each layer. See Table 1 for the used functions and their kinetic parameters.

As indicated above, bulk SOC stocks reached an equilibrium in the 0-30 cm layer. In contrast, bulk SOC stocks in the deepest soil layer (30-50 cm) were still decreasing at a rate of 0.25 Mg C ha^{-1} yr^{-1} ± 0.04 (R^2: 0.71; Table 1) after 56 years. This finding is of interest as no studies have reported, so far, effects of land use change to OP in subsoil horizons. The obtained C loss rates for bulk SOC stocks (slope1 for three uppermost soil layers and linear regression slope for the deepest layer) decreased gradually with soil depth from 0.42 Mg C ha^{-1} yr^{-1} in the surface 10 cm, to 0.13 Mg C ha^{-1} yr^{-1} in the 30-40 and 40-50 cm soil depth (calculated from half of the depth 30-50 cm).

2.4.2 Ecosystem C stocks

Oil palm plantations contain substantially greater total biomass (above and belowground) than pastures. Total OP biomass increased at an accumulation rate of 3.3 ± 0.1 Mg C ha^{-1} yr^{-1} along the 30 years of both, the first and the second OP cultivation cycle. This corresponded to a time-averaged OP biomass C stocks of 49.5 ± 1.5 Mg C ha^{-1}, i.e. 5 times more than 10 Mg C ha^{-1} typically found in the pastures of this region (Braz et al. 2013; Mosquera et al. 2012). Although the SOC stock varied with plantation age, in general soil was the largest C pool in the ecosystem. The contribution of SOC to TEC stocks ranged from 38% in the 30-year old first cycle plantation, to 87% in the recently replanted plantation 2-year old second cycle plantation (32 years).

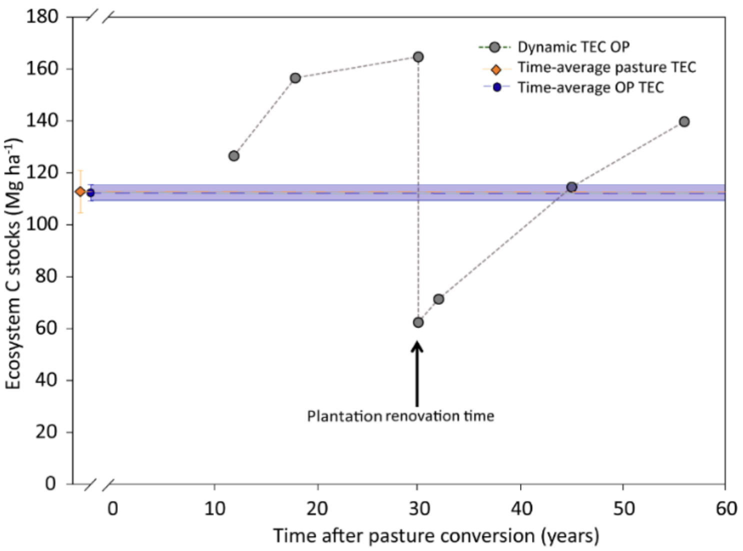


Figure 3. TEC stocks. TEC in OP plantations and pastures includes above- and belowground biomass and SOC stocks down to 50 cm (but not dead trees after replanted). The orange diamond and its vertical SE bars correspond to the time-average TEC stocks in pastures. Purple circle and its vertical SE bars correspond to time-average TEC stocks in OP, and purple band indicates the time-average TEC stocks during the 56 years of OP cultivation.

Time-averaged TEC in pastures reached 112.8 ± 8.3 Mg C ha^{-1}, assuming constant SOC stocks (102.8 ± 8.3 Mg C ha^{-1}) and a total pasture biomass of 10 Mg C ha^{-1} (Fig. 3). Over a large number of OP rotation cycles, time-averaged SOC stocks in OP plantations would be equal to the stocks at equilibrium (62.61 ± 2.73 Mg C ha^{-1}; Fig. 1). Accordingly, time-averaged TEC in OP plantations reaches 112.3 ± 3.2 Mg C ha^{-1} (Fig. 3). This indicates that the conversion of pastures to OP plantations in this region is close to C neutrality (-0.5 ± 8.8 Mg C ha^{-1}) considering multiple OP cycles. The rate of C accumulation in the biomass was higher (3.3 ± 0.1 Mg C ha^{-1} yr^{-1}) than the rate of SOC losses (1.26 ± 0.3 Mg C ha^{-1} yr^{-1}). As a result, TEC during the first rotation cycle were always higher than in pasture, but only about half of the time for the subsequent second rotation cycle when SOC stocks have reached equilibrium (Fig. 3). Therefore, OP plantations were continuously a C sink, even during the phase of SOC losses, except at the time of plantation replanting when OP biomass is destroyed.

2.4.3 Soil fertility

SOC content, unlike SOC stocks, increased significantly after 41.1 ± 2.7 years following an initial decline in the 0- to10-cm. However, this C recovery did not reach the initial soil C content present under pasture (2.5 ± 0.1%, Fig. 4A). The rate of increase in soil C content was almost the double as compared to the decrease (-0.03% ± 0.02). This partial recovery of SOC content was observed down to a depth of 30 cm but not below where SOC content was still decreasing linearly (R^2= 0.75) after 56 years of OP cultivation.

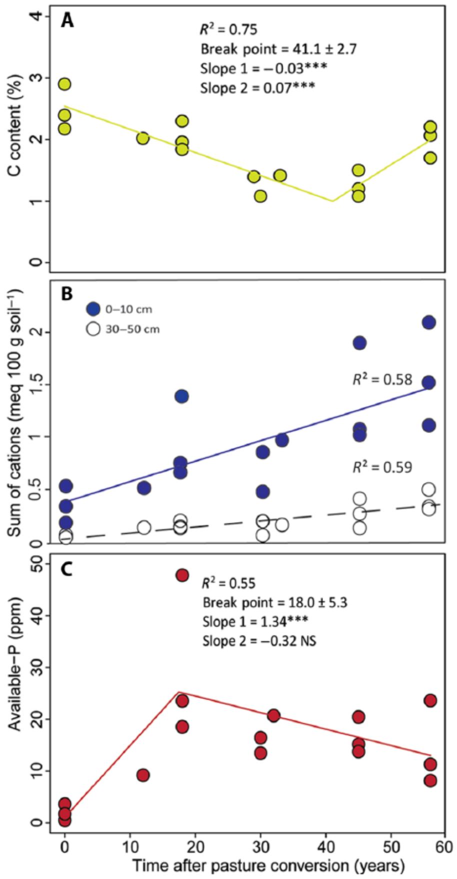


Figure 4. Soil chemical fertility dynamics. (A) Soil C content as a function of time after pasture conversion into OP plantations at the soil surface (0- to 10-cm). The yellow line represents the fitted segmented regression equation. Significance of the slope coefficients from each side of the breaking point is indicated (****p* < 0.001). **(B)** Sum of cations as a function of time after pasture conversion into oil palm plantations at two soil depths: close circles 0- to 10-cm and open circles 30-to 50-cm. The lines represents the linear regression equations. **(C)** Available Phosphorus (Bray-P) as a function of time after pasture conversion into oil palm plantations at the soil surface (0- to 10-cm). The red line represents the fitted segmented regression equation. Significance of the slope coefficients from each side of the breaking point is indicated (****p* < 0.001).

Macronutrient availability was strongly enhanced down to 50-cm depth (Table S2). The sum of cations showed a decreasing trend with depth at all sites. Nonetheless, all layers exhibited a similar enrichment factor of 3.9 to 5.6 of the sum of cations, indicating nutrient leaching of the applied nutrients by fertilization. Nutrient enrichment did not show any saturation with time (Fig. 4B). Base saturation increased in the four layers, up to a factor of four 56 years after conversion in the 30-50 cm depth layer. Available Bray P showed a different pattern. It peaked at 18.0 ± 5.3 years and then levelled off at higher levels than in pastures in the top 10 cm (Fig. 4C). Interestingly, this same pattern with small variations in the estimated breakpoint was found for the 30-50 cm soil depth. However, a significant decrease during the last years of the first cycle and the entire second cycle followed the observed peak. This suggests leaching of P into the subsoil in the short-term (first OP cycle). Soil pH values exhibited a narrow range from 4.0 to 4.6 across land uses and soil depths.

Oil palm cultivation did not have a constant effect throughout the two rotation cycles and soil depth as shown by PCA (Fig. 5). During the first cycle the amount (SOC content) and quality (C/N ratio) of OM decreased while the nutrients and bulk density increased in the surface (0- to 10-cm) and subsoil (30- to 50-cm) layers as shown by the first principal component (PC1, 35.7% at 0-10 cm and 45.1% at 30- to 50-cm). During the second cycle, the effects of cultivation remained similar in the subsoil (Fig. 5B) while the surface soil layer showed a partial recovery of SOC content, but not SOC quality, suggesting a slight improvement of topsoil fertility in the oldest plantations.

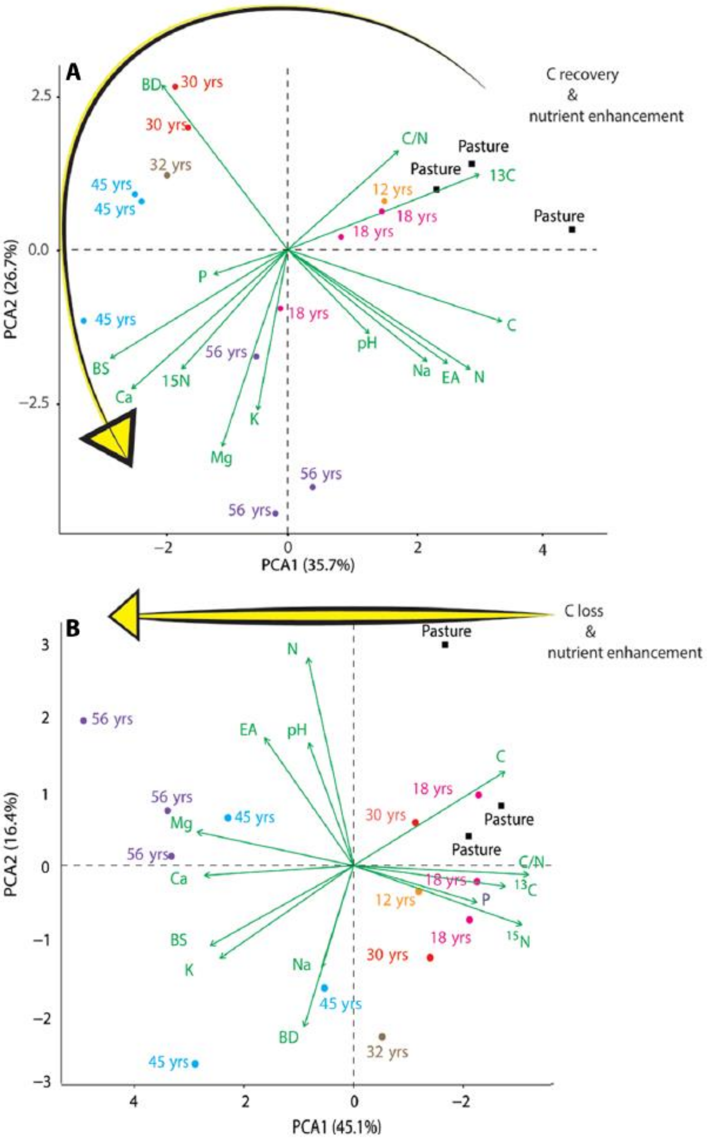


Figure 5. PCA of soil properties in pastures and OP plantations derived from pastures. (A) the soil surface (0- to 10-cm, (B) the subsoil (30- to 50-cm). BD, bulk density; C:N, C and N ratio; ^{13}C, δ^{13}C; C, carbon; N, nitrogen; EA, exchangeable acidity; Na, sodium; pH; K, potassium; Mg, magnesium; ^{15}N, δ^{15}N; Ca, calcium; BS, base saturation and P, Available phosphorus (Bray P). The OP plots are indicated with time after conversion in years (yrs).

2.5 Discussion

We demonstrate that the conversion of pasture-to-OP in Colombia compares favorably in terms of ecosystem C changes to that of OP expansion into forested lands as often occurs in major OP producing countries in SE Asia. While the conversion of rainforests in Sumatra (Guillaume et al. 2018) led to a loss of 173 Mg C ha^{-1}, the conversion of pasture-to-OP plantation was C neutral (-0.5 ± 8.8 Mg C ha^{-1}), reducing TEC losses by 99.7 ± 9.6%. In addition, establishing OP on pastures not only avoids the large initial loss of C stored in rainforest biomass, but also increases biomass C pools. Nonetheless, converting pastures to OP did not act as C sink because the conversion caused large SOC reductions that counterbalanced the gains in OP biomass C.

The time-averaged C stored in OP biomass is mainly influenced by the length of rotation cycles (Guillaume et al. 2018). Shortening the rotation time to 25 years, as common in SE Asia, would reduce the time-averaged biomass C by 17% (about 8 Mg C ha^{-1}), leading to a small C loss following land use change. Oil palm biomass C found in Colombian plantations was similar to those reported for Indonesian's (Guillaume et al. 2018; Khasanah et al. 2015). Carbon pools that were not measured in this study, such as frond piles or understory vegetation if present (< 3 Mg C ha^{-1}), have a negligible contribution to TEC (Khasanah et al. 2015). The main factor determining if the land use change will result in small C gains or losses is the initial amount of SOC in the converted grassland ecosystem. Indeed, SOC losses after land use change are not constant but rather proportional to the initial SOC stocks (van Straaten et al. 2015). The initial variability of SOC stocks in the studied pastures (standard deviation of 14.3 Mg C ha^{-1}) surpassed alone the C stored in grass biomass. The managed pastures investigated in this study could represent the land use type with the highest initial SOC storage on well-drained soils in this region, as *Brachiaria* pastures favor soil C sequestration when they replace either forested or grassland areas in the Neotropics (Cerri et al. 2007; Fisher et al. 2007). Also, because the rainfall and rainy season length are higher, soils in the piedmont often harbor slightly higher C stocks than the savannas further to the east. Lower SOC losses, and thus a slightly positive change in ecosystem C storage, are therefore expected when OP plantations are established on degraded pastures or other part of the Llanos (Jiménez and Lal 2006). The same argument is often used to justify the transformation of the native savannas, without due regard to the biodiversity and ecosystem service values of these natural ecosystems. Given that the Llanos is expected to remain an important OP expansion area in Colombia, special attention needs to be given to the loss of native savannas as opposed to pastures.

The amount of SOC lost after the conversion of these pastures was higher than the ones reported when rainforest were converted to OP plantations, likely because of the high amount of SOC (102 Mg ha^{-1}) stored down to 50 cm in the studied pastures (Chiti et al. 2014; Guillaume et al. 2015; van Straaten et al. 2015). This finding is in line with those previously found in a Brazilian study, where SOC losses were more pronounced for OP plantations derived from pasture than from forested areas (Frazão et al. 2013). However, the proportion of initial SOC lost in this study (39%) was similar to the proportion reported for rainforest-derived plantations with the difference that it took longer to reach a new equilibrium in the pasture-derived plantations (van Straaten et al. 2015).

The dynamics of SOC stocks after land use change is commonly assumed to follow a single exponential decay until it reach a new equilibrium, i.e. most losses occur within a decade after conversion (de Blécourt et al. 2013; Lobe, Amelung, and Du Preez 2001; van Straaten et al. 2015). In this study, losses were constant and extended over a longer time. The initial sharp decrease followed by a stabilization phase confirms the slow response of SOC to land use change and highlights the importance to assess changes in C cycling dynamics either over longer timescales or to investigate more sensitive SOC indicators, i.e. particulate organic matter, than total SOC pool size when only plantations within the first rotation cycle are studied. The dynamics of total SOC stocks was the net result of distinct patterns of C pool dynamics depending on soil depth and C origin. The decomposition of pasture-derived SOC did actually follow a single exponential decay in each layer, while OP-derived SOC accumulated at constant rate, except for 0-10 cm depth where SOC accumulation reached saturation. Despite the apparent stabilization of total SOC stocks down to 50 cm during the second rotation cycle, the isotopic approach demonstrated that only the top 10 cm layer was close to the equilibrium level (Fig. 2A). Between 10-30 cm depth, rates of pasture-derived C loss and OP-derived C stabilization were similar in the oldest plantations, so that bulk stocks appeared to have reached equilibrium. The decomposition rate in the 30-50 cm layer was still faster than the stabilization rate, explaining that the subsoil was still losing C after 56 years of OP cultivation. This finding is of relevance as no studies have reported, so far, effects of land use change to OP in subsoil horizons, presumably due to the relative short-term duration of the existent literature.

It is yet not possible to determine at which level SOC stocks will stabilize for layers below 10 cm depth since the accumulation of OP-derived C did not show any sign of saturation even after 56 years. Under certain conditions, SOC stocks reach a minimum before increasing again, as the common U-shaped reported in other long-term SOC dynamics studies (Covington 2011; Zak et al. 2011). Because the rate of pasture-derived SOC losses decreased with time after conversion, stabilization rates can surpass decomposition rates if the stabilization does not saturate before. This mechanism can explain the recovery of C content in the top 10 cm (Fig. 4A) and would confirm the similar tendency observed for SOC stocks at least down to 20 cm depth (Fig. 2, A and B). It is commonly observed that SOC turnover slow down with increasing soil depth because of higher level of SOC protection by minerals, reduced microbial activity or reduce roots C inputs (Balesdent et al. 2018). Because of slower C dynamics in deep soil, it is possible that layers below 10 cm depth will reach the recovery phase observed in the topsoil in the third rotation cycle. Nonetheless, it is questionable if the soil below 30 cm depth will follow the same dynamics as the upper layer to eventually reach this stage (Fig. 5B). Oil palm rooting system has a specific architecture with fine roots growing upward to the surface from exploratory coarse roots growing horizontally at around 30 cm depth (Jourdan and Rey 1997). Hence, root biomass and activity are greatest in the top 30 cm. Layers below 30 cm receive less C inputs from roots, but are still strongly enriched with nutrients leached from the heavy fertilization, favoring SOC mineralization. Consequently, in spite of the stabilization of SOC stocks during the second rotation cycle, stocks might slightly increase or decrease in subsequent rotation cycles depending on the intensity of the recovery in the upper layer and the stabilization level below 30 cm depth.

Our findings demonstrate that the conversion of pasture into OP had an important impact on SOC stocks. The most important long-term changes in SOC storage occurred during the first OP rotation cycle where 39% of the original SOC was lost. The second phase was characterized by a redistribution of SOC within the soil profile, in which a recovery of SOC in the topsoil compensates for a decrease in the subsoil, leading to a stabilization of the total SOC stocks down to 50 cm depth. The negative impact of OP on SOC storage was high when cultivated on pastures, but it was comparable to the impact of intensive cultivation of other non-perennial crops on pasture or rainforests (Don et al. 2011; Guo and Gifford 2002). Soil degradation was mainly limited to a decrease of SOM. The typical threats affecting soil physical quality in OP plantations established on rainforest such as erosion and compaction where limited in the studied plantations (Guillaume, Maranguit, et al. 2016; Guillaume et al. 2015). Even though, the reference pasture soils might have been compacted by trampling, native savanna soils in the Llanos region are naturally compacted and thus less sensitive to further compaction(Lavelle et al. 2014a). Also, the topography of the Llanos region is mostly composed of flat areas (i.e. piedmont and flat high-plains) reducing the risk of soil erosion, contrasting with Indonesian islands where plantations are affected by severe soil erosion (Gharibreza et al. 2013; Guillaume et al. 2015).

Because of the soil preparation prior OP establishment and frequent fertilizers applications, inherent with low soil quality of the local soils, soil physical and chemical fertility remained similar or even higher after two OP cultivation cycles. Specifically, while SOC declined, the sum of exchangeable bases increased during the pastures to OP transition, implying a tradeoff between soil chemical properties and SOC in the long-term. This suggest that OP nutrient supply relies mostly on the frequent application of mineral fertilizers and not on nutrients released from SOM mineralization by microorganisms. Nonetheless, soils did not show signs of degradation that would prevent the establishment of new crops or the restoration of (semi-)natural plant communities following OP cultivation. This is fundamental because TEC changes remain theoretical since it assumes that OP cultivation will last permanently. Ultimately, the impact of land use change will depend on the land use following OP. Since biomass C stocks gains disappear rapidly while SOC losses remain, the following land use type would start with a C debt of about 40 Mg C ha^{-1} in the soil after 60 years of OP cultivation. It is, therefore, important to maintain soil physical and chemical properties because SOC losses from land use change are, to a high degree, reversible (Don et al. 2011). The sustainability of OP cultivation would, therefore, benefit from the implementation of management practices that incorporate organic residues, either as empty fruit bunches, compost, or by using cover crops, into the regular soil management programs.

Importantly, soils under OP plantations in this study were far from C-saturation, as indicated by the lower SOC stocks than those found in pastures and by the linear increase of OP-derived C observed in subsoil layers. Therefore, a large potential for C sequestration in soils subjected to this land use change alternative can be expected if OM inputs increase. Furthermore, increasing SOC and nutrients in an organic form limits the tradeoff that negatively impacts soil biota by increasing synergies between soil chemical properties and SOC (Tao et al. 2016; Tao et al. 2017). Replacing soil biological activity by mineral fertilization is risky if fertilizer costs increase or if their supply decreases in the future. Moreover, soil biota improve soil stability and resilience, facilitating the restoration of former OP plantations (Bender, Wagg, and van der Heijden 2016).

Pastures tend to emit more nitrous oxide emissions (N_2O) than tropical forests (Van Lent, Hergoualc'H, and Verchot 2015), therefore if OP will expand on pastures it is plausible that this alternative land use change will compare positively to the common deforestation scenario in terms of N_2O emissions. Adoption of a suite of practices for better nutrient management, i.e. customized fertilization programs, should be encouraged in OP plantations not only to reduce mineral nutrient losses but also to limit their stimulating effect on SOM decomposition in subsoil layers, favoring deep SOC depletion, and reduce the greenhouse gas emission budget from preventing N_2O emissions. Oil palm is blamed for its large environmental impacts, especially the reduction of C stocks and biodiversity from tropical forest conversion, so the search for low-impact land use change alternatives has become imperative. This study provides for the first-time empirical field-based evidence that the conversion of pastures to OP is C neutral from an ecosystem C storage perspective. The availability of large pasture areas in the tropics, particularly in Latin America, could limit the negative impacts of ongoing OP expansion to native savannas and natural forests (Furumo and Aide 2017; Vijay et al. 2016). Our findings indicate that conversion of pastures to OP can be an opportunity to preserve and even increase C sequestration in the tropics, and reduce the large C footprint of OP development on forested land. Recent studies on biodiversity in the Neotropics have also demonstrated that OP plantations established on pastures can spare the development of other ecosystems that are much richer in endemic and threatened vertebrates (Ocampo-Peñuela et al. 2018; Prescott et al. 2016).

The spatial design of OP plantations can also take greater account of other natural land cover elements, including remnant forests and savannas. This more heterogeneous landscape mosaic would likely provide increased ecosystem resilience favoring both productivity and conservation. We hope our study will prompt research on other fundamental aspects of OP expansion in pastures areas, such as seasonal water scarcity and socio-economic aspects. We recognized that a shift in OP expansion from forested land to unproductive pastures will need to be supported by policy. Improving support services, including infrastructure development such as mills and transport facilities, as well as facilities for the substantial labor requirements that will be required, could encourage the development of OP in regions where there are unproductive pastures. Further measures like banning forest conversion or, on the consumer side, demanding effective certification schemes for OP production would also reduce deforestation for OP expansion and its dramatic environmental impacts

3 Manuscript 2: Deforestation free expansion of oil palm and improved management to reduce C footprint

*“ **Daring ideas** are like **chessmen** moved **forward**; they may be beaten, but they may **start** a **winning game**”*

Field work in Los Llanos of Colombia

Juan Carlos Quezada Thomas Guillaume, Christopher Poeplau, Alexandre Buttler

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3.1 Abstract

Land use change (LUC) and subsequent intensive agricultural management in the tropics are associated with detrimental impacts on carbon sequestration and agroecosystem resilience. While various deforestation-free strategies with improved management practices have been proposed to enhance oil palm (OP) sustainability, field-based evidence is remarkably scarce. Here, we show that clayey-soil savanna conversion into OP increased total ecosystem C stocks by 40 ± 13 Mg C ha^{-1} during a full OP cultivation cycle of 27 years. Distinct management within the plantation created high spatial and temporal heterogeneity of soil characteristics. Organic residues amendments triggered the accumulation of soil organic carbon by up to 1.9 Mg ha^{-1} yr^{-1} over the full rotation cycle. Besides, organic amendments sustained greater soil biological activity than high mineral fertilizer inputs, corresponding to similar level as in savannas. Surprisingly, management practices explained nearly five times more variability of soil biogeochemical properties than the LUC *per se*. Our findings highlight a clear opportunity to bring OP cultivation closer to the path of sustainability by incentives designed to favor improved management practices.

Keywords: Land-use change, soil organic carbon, soil fertility, biomass, savanna, δ^{13}C, ecosystem C stocks, C sequestration

3.2 Introduction

Understanding the impacts of land use change (LUC) and subsequent agricultural management in the tropics is of increasing prominence given their frequent adverse consequences on the environment and climate (Harris et al. 2012; Röll et al. 2019; Wilcove et al. 2013). Owing to the vast expansion of agriculture on previous forested areas and the global importance of forest ecosystems, much work related to deforestation has been undertaken. Tropical deforestation is associated with losses in ecosystem C storage of ~60% when perennial plantations are established and this contributes to climate change and poses challenges to achieve sustainability in agroecosystems (Guillaume et al. 2018; Ziegler et al. 2012). Soil C storage is usually reduced (Don et al. 2011; van Straaten et al. 2015), with a concomitant decline in soil fertility and biological activity that cast doubts on the soil capacity to sustain OP cultivation on the long-term (Guillaume, Maranguit, et al. 2016; Kooch, Tavakoli, and Akbarinia 2018; Kopittke et al. 2017; Maranguit, Guillaume, and Kuzyakov 2017).

For all these negative impacts, expansion of agriculture into forested ecosystems needs to be slowed down and ultimately ceased. Various less impactful LUC scenarios have been proposed, but scant evidences exist on the effectiveness of these strategies. From an ecosystem C standpoint, considering soils and vegetation, alternatives should include the replacement of low-biomass land with low soil C by highly productive and high-biomass perennial plantations (Gibbs et al. 2008; Ziegler et al. 2012). In this context, alternatives to avoid deforestation include the conversion of degraded land (*i.e.* degraded pastures) or other non-forested natural ecosystems like savannas into oil palm (OP) plantations (Goodrick, P. N. Nelson, et al. 2015; Quezada et al. 2019). For example, in South-America there are initiatives to decouple the expansion of agriculture from deforestation: since 2017, there is a zero-deforestation agreement for palm oil production between the Colombian government and the private OP sector (TFA 2020 2017). This kind of multi-sectorial efforts attempts to address the international and general persistent concerns about the expansion of OP at high environmental cost.

Expansion of OP in Colombia has shown to be at minimal deforestation cost (Furumo and Aide 2017; Vijay et al. 2016), which opposes the large-scale forest clearance in SE-Asia. A large share of the total expansion of OP in Colombia has occurred in the non-forested savanna region of Los Llanos in eastern Colombia and future expansion in this area is predicted to continue (Castiblanco et al. 2013; Etter et al. 2010). Savannas ecosystems are characterized by a suite of conditions that in the past were seen as not suitable for agricultural production. In general, the Colombian savannas are of acidic soils with poor nutrient and soil organic matter (SOM) contents and high aluminum toxicity (Basamba et al. 2006; Guimarães et al. 2004). Paradoxically, such inherently poor savanna soils are also often cited as the last frontier for agricultural expansion given their vast territories in the tropics and some positive characteristics for large scale agriculture, *i.e.* flat topography (Ayarza M; Barrios E; Rao IM; Amézquita E; Rondón M. 2007; Guimarães et al. 2004; Rudel et al. 2015). In the current midst of global climate crisis, the transformation of savannas into OP agriculture could provide an important climate change mitigation option not only for sequestering C in the biomass, but also through soil C sequestration (Laganière, Angers, and Paré 2010; Sanderman, Hengl, and Fiske 2017). Coupled with global warming mitigation, enhanced soil fertility is an expected co-benefit of savannas conversion if enhanced management practices are applied like adding crop residues (Rhebergen et al. 2020; H. H. Tao et al. 2016; Tao et al. 2017).

Following savanna conversion into new agricultural land, the subsequent management will become the main driving factor determining the direction, magnitude and rate of change of a wide range of soil properties and processes including nutrient cycling, carbon storage and biological activity. Fine-scale shifts in soil properties are common in OP plantations due to the well-defined and contrasted management imposed over long time periods, for example with the accumulation of OP crop residues in between palms and nutrients in specific management areas within the plantations (Rüegg et al. 2019). This heterogeneous distribution of practices and resources within plantations have to be considered when proper assessments of soil properties and processes are attempted (Nelson et al. 2015). With cultivation time, the constant use of specific carbon and nutrient inputs in certain areas creates the so-called management zones (MZs) that start to be visually recognizable in mature plantations of around 6-8 years. Management zones include: the weeded circle (W), a circular bare surface close to the OP trunk of around four meters diameter, where fertilizers are exclusively applied in early plantation development (1-5 years old); the frond pile (F), where almost all the aboveground C inputs in OP plantations occurred by stacking pruned fronds and also some fertilizer is added when plantations are between 6-30 years; every second path between OP rows is defined as a harvest path (H), this is a bare soil area where farm machinery circulates and from where fertilizers are evenly spread in mature and old plantations (6-30 years) to W, F and the interzone (IZ), which is the between rows spaces, that alternates with the H zone and where understory vegetation grows sporadically (Supplementary Fig. 1). Such within plantation heterogeneity between MZs offers an ideal study system for robust assessments of the responses of soil properties and processes to different and consistent management practices.

Soil properties and processes between OP plantations MZs are different. This includes variations in the spatial distribution of nutrients, macrofauna, earthworms and physical properties (Bakar et al. 2011; Carron et al. 2015, 2016; Guillaume, Holtkamp, et al. 2016; Tanaka et al. 2009; H. H. Tao et al. 2016). Other more recent studies found highly variable C and N stocks between MZs, with the F area being the one with the highest SOC stocks (Frazão et al. 2013, 2014; Ni'matul Khasanah et al. 2015; M. C. Law, S. K. Balasundram, M. H. A. Husni 2009). Although the impacts of management practices in OP plantations on soil physico-chemical aspects have been widely studied, information on their effects on the processes influencing SOC stabilization is limited. It is known that tropical forest conversion to OP changes the composition of bacterial and fungal communities (Kerfahi et al. 2014; Lee-Cruz et al. 2013; Tripathi et al. 2016), whereas grassland conversion to OP did not change microbial community composition (Wakelin et al. 2016). However, a limitation of the only study on grassland to OP conversion characterizing soil microbial composition (Wakelin et al. 2016) is that it excluded samples from other MZs than the IZ. In that work, the authors suggested that soil microbial community composition could be more responsive to the variability of soil physico-chemical properties in the MZs, than to the LUC *per se*. More recently, the role of management practices, mediated by soil microorganisms, on SOC stabilization in savanna conversion to OP was evaluated in a 9-year old plantation (Rüegg et al. 2019). It was shown that the main controlling factor of SOC stabilization was soil C inputs through OP fine roots, which in turn were directly affected by nutrient addition and mediated the effects of soil microbes. Although, it is clear that management practices exert a direct control on SOC stabilization, it remains inconclusive how the cumulative effects of management practices affect SOC stabilization with time after savanna conversion, *i.e.* effects of cumulative OM or nutrient inputs in old plantations. The findings in (*29*) also suggest that the aboveground added crop residues were not as efficient as the belowground inputs, fine roots mainly, to stabilize SOM. Even though there has been some progress in elucidating the mechanism that drives SOC stabilization in savanna conversion, the temporal patterns of soil microbial activity and how this affects SOC stabilization remains largely unknown. At present, estimates of SOC cycling from savanna conversion into OP is limited because almost all research work has been focused on the common deforestation LUC to OP. In this context, recent work has shown that pastures conversion, from former savanna areas, into OP can be C neutral at the ecosystem level (Quezada et al. 2019). Therefore, it is highly relevant to assess the environmental impacts of direct savanna conversion into OP. In this study, we address the above-mentioned knowledge gaps and assess the impacts of direct savanna conversion into OP over a time frame that encompasses a full rotation cycle.

The overarching aim of this study was to assess the effect of savanna conversion to OP, as a deforestation free alternative, on ecosystem C storage and to explore how management practices could improve the C footprint of this LUC type. Consequently, we quantified changes in ecosystem C stocks, including the C pools in the vegetation and in the soil, down to 70-cm, using a chronosequence of OP plantations that expand over a time frame of a full OP cultivation cycle (27 years). We expected to obtain a positive ecosystem C balance in the savanna derived OP system given the higher biomass in OP than in savanna vegetation. Furthermore, using the gradual change in the ^{13}C soil signature that the savanna (C4-vegetation type) into OP (C3 vegetation type) LUC involves, we aimed to further increase our understanding on SOC dynamics under different management practices. Depending on MZ, we expected to observe different directions and rates of changes in the accumulation of new OP-derived C and the decomposition savanna-derived C, with highest SOC-sequestration rates in zones of crop residue accumulation (F). Finally, we also aimed to characterize changes in soil biogeochemical properties for each MZ. For this, we hypothesized that along with increases in soil chemical fertility, soil biological properties including enzymatic activity, microbial biomass, soil respiration and others in areas with high crop residues additions will also be enhanced.

3.3 Material and methods

3.3.1 Study area and design

The study area was located at ~20 km east of Puerto Gaitán in the Department of Meta, Colombia, in the slightly undulating well-drained high plains savannas region of Los Llanos (Altillanura plana). The region experiences a tropical climate (mean annual temperature of 26 °C and yearly precipitation of 2200 mm yr^{-1}) with a distinct dry season from December to March and 95% of the yearly rain falling between April and November (Lavelle et al. 2014b; Rippstein et al. 2001). Soils are dominated by Plinthosols and Ferralsols (IUSS Working Group WRB 2014). Low fertility, high acidity and high aluminum saturation limiting agricultural production are predominant characteristics of these savanna soils (Lavelle et al. 2014b; Rippstein et al. 2001). The savanna vegetation is characterized by C4 tropical grasses (mainly *Andropogon* and *Trachypogon* grass species) and small scattered patches of fire-resistant shrub species. All selected OP plots were within the large-scale commercial farm of "Sapuga" (4° 3'49.8"N, 71°59'38.4" W) of about 3,000 hectares. The two reference savanna sites were in the vicinity of Sapuga farm. The OP sites were selected with the aim of evaluating changes in soil characteristics following savanna conversion and therefore they were carefully chosen with the help of plantation agronomists to ensure that the investigated plantations had been established on unmanaged native savannas, *i.e.* no cattle grazing in the past. A total of four OP plantation sites were identified, with time after savanna conversion being 8-,12-, 23- and 27-years. Also, to minimize biased estimates by variations in soil clay content, all the study sites were taken on clayey soil of between 38 to 48% clay content. The fertilization regime included two applications per year during the early and middle stages of rainfall periods. At plantation establishment, 2-3 tons of a mix of lime dolomite with phosphoric rock are applied. Throughout the plantation life span, between 150 to 600 kg of NPK alongside with some micronutrients like magnesium, boron and zinc are given. For example, in mature plantations of this study, between 8-23 years, the usual application rates were of 128 kg of N ha^{-1} yr^{-1}, 42 kg of P_2O_5 ha^{-1} yr^{-1} and 300 kg of K_2O ha^{-1} yr^{-1}.

3.3.2 Soil sampling and sample preparation

At each OP site, a 1-hectare plot was selected. Plots were representative of the plantations in this region (*i.e.* soils, management, fertilization) and plant density was 144 palms ha^{-1}. Within each plot, 10 palm trees were randomly selected for referencing soil sample collection. Soils were sampled in the four MZs described before around each selected palm (see Supplementary Fig. 1). Samples were collected with a 6-cm diameter corer to a depth of 70 cm, with intervals of 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, 30-50 cm and 50-70 cm. This resulted in 24 samples at each OP site. Savanna sites were sampled along a 100-m long transect with five evenly spaced sampling points. As MZs do not occur in savannas, final samples were pooled by depth, six, for each of the two sampled savannas. After field collection and before further analyses, soil samples were homogenized, air-dried, sieved through a 2-mm stainless steel sieve and stored in plastic bags at room temperature until transportation to the laboratory in Lausanne, Switzerland. For soil chemical analysis, samples were oven-dried at 35°C and analyzed within ~100 days after field collection.

3.3.3 Aboveground biomass carbon stocks

Aboveground biomass (AGB) estimations were conducted by measuring the heights of the ten randomly selected palms within the 1-hectare plot from the palm base to the base of the youngest fully expanded leaf (Kotowska et al. 2015). Then aboveground biomass estimation was done with the use of the allometric equation (Khasanah et al. 2015):

AGB OP (Mg C ha^{-1})= 0.0923 * height [m] + 0.1333

Belowground biomass (BGB) in OP was estimated though allometric equation (Göttingen and Syahrinudin 2005) :

BGB OP (Mg C ha^{-1})= 1.45 * age + 9.88 * 144 palms ha^{-1}

A factor of 0.413 was used to convert AGB and BGB to estimate biomass C stocks (Mg C ha^{-1}) (Göttingen and Syahrinudin 2005).

For savannas AGB measurement, herbaceous vegetation was sampled from one square meter in triplicates in each savanna, close to the transect where soils were sampled. Savannas BGB was sampled at five sampling points in each savanna with a corer of 5-cm diameter to a depth of 30 cm. Roots were separated from the soil by sieving at 2 mm and rinsing. Dry BGB was determined after 48 hours of drying at 60°C. Other ecosystem C components such as litter layer in savannas were negligible (Batlle-Bayer, Batjes, and Bindraban 2010). All biomass measures are given in Mg C ha^{-1}. Total biomass time-averaged C (AGB + BGB) in OP plantations was estimated as the stocks accumulated in half the time of a rotation cycle in a 30-year plantation as the increase of OP biomass is linear. Time-averaged TEC stocks resulted from summing the total time-averaged biomass and mean soil C stocks to a depth of 70 cm across all sites. Mean soil C stocks across sites was used because no change over cultivation time were detected.

3.3.4 Laboratory analysis

Soil particle size was determined by the pipette method after removal of the organic fraction with 30% H_2O_2 (G. W. Gee 1986). Soil pH was measured in water on a 1:2.5 (soil:water) suspension with a glass electrode. Soil available P was determined according to Bray II (Bray, R.H. and Kurtz 1945) and P in the extracts was determined colorimetrically. Available cations including Ca, K, Na and Mg were analyzed with Mehlich-III solution (Mehlich 1984) and horizontal shaking for 5 min followed by inductively coupled plasma spectrometry (PerkinElmer, Waltham, Massachusetts, USA). Exchangeable acidity (EA) was determined extracting 2 grams of dry soil with 10 ml of 1 M KCl, on a reciprocal shaker for 30 min. The extracts were then allowed to settled for 30 minutes and filtered three times with 30 ml of 1 N KCl. After adding phenolphthalein, final extracts were titrated with 0.01N NaOH. Effective cation exchange capacity (ECEC) was obtained by summing the amount of charge per unit soil (meq

100 g^{-1}) of major cations (Ca, K, Na, and Mg) and exchangeable acidity. Base saturation resulted from the division of the total sum of charge per unit soil of Ca, K, Na, and Mg by ECEC. Total C and N contents, ^{13}C and ^{15}N were measured on oven-dried at 35°C and ground soil encapsulated in tin cups with an Elemental Analyser (Eurovector) coupled to an isotope ratio mass spectrometer (Delta plus, Thermo Fisher) at the Center for Stable Isotope Research and Analysis (KOSI) of the University of Göttingen. Samples were free of carbonates and therefore total C content is equivalent to SOC.

Soil biological analyses were conducted on top- (0-5 cm) and subsoil (50-70 cm) samples of three MZs (W, IZ and F). Samples of the H zone were not considered due to the similar chemical conditions that they shared with IZ samples. Measured microbial parameters included microbial biomass C (MBC), microbial biomass N (MBN), basal respiration, potential activity of four extracellular enzymes, dissolved organic C (DOC), ammonium (NH_4) and nitrate (NO_3). Analyses were conducted following a 7-day incubation period in the dark. Twenty grams of sieved and oven-dried soils were rewetted to reach 60% water holding capacity and incubated at 24°C in 250-ml glass bottles closed with rubber septa.

Basal respiration measurements were performed by injecting air samples taken from the headspace air in the glass bottles with an airtight syringe into an infrared gas analyzer (EGM-4 PP Systems, Amesbury, Massachusetts). For this, the instrument's static sampling mode was set. Following headspace samples collection, CO2-free air was flushed into the glass bottles. Soil MBC and MBN were determined on the incubated soil with the chloroform fumigation-extraction method using an extraction coefficient of 0.45 for calculating MBC (Beck et al. 1997) and of 0.54 for MBN (Brookes et al. 1985). The DOC derived from soluble extracted C with K_2SO_4 on the non-fumigated samples. For extraction of inorganic N (NH_4 and NO_3), 5 grams of incubated soil were extracted with 30 ml of 1M KCl and measured with an automated analyzer (SEAL AA3 HR Autoanalyser) and results expressed as mg kg^{-1} oven dried soil. To estimate soil microbial functioning, indices of single indicators were calculated. The microbial quotient was estimated as the ratio of MBC to SOC and the metabolic quotient as the ratio of basal respiration to MBC. Potential enzyme activities involved in the degradation of C, N and P including those involved in degrading chitin (β-1,4-N-acetylglucosaminidase), leucine amino acid (leucine aminopeptidase) and phosphate groups (acid phosphatase) were assayed by fluorimetric microplate assay using a modified method (Marx et al. 2005). Briefly, 1 g of incubated soil was mixed and homogenized for 1 minute with a Vortex in 50 ml of milli-Q water and then sonicated at 50 J s^{-1} for 2 minutes (Ultrasonic Disintegrator). All assays were performed in buffered conditions with 2-N-morpholino-ethanesulfonic acid (MES) and Trizma for 4-methylumbelliferone (MUB)- and 7-amino-4-methycoumarin (AMC)-based substrates, respectively. For each sample three aliquots of 50 µl of the stirred suspension were mixed in a 96-well microplate with either MES or Trizma buffer and 100 µl of substrate solution of increasing concentration. After 0.5 and 2 h of incubation in the dark, the fluorescence was measured on a fluorometric microplate reader (BioTek SynergyMX). To quantify product release and account for quenching effects, a set of standards was prepared using MUB and AMC mixed with soil extract. Enzyme activities were expressed as nmol of substrate (MUF and AMC) converted hour^{-1} and g^{-1} (dry weight) of soil

3.3.5 Soil C stocks

The calculated SOC stocks resulted from the product of soil bulk density, layer thickness and SOC concentration. Soil bulk density samples were collected down to 70 cm depth in one soil pit at each 1-hectare plot. The pit was dug in the IZ area given the large proportion that it represents at the plot level (Table 1). Two stainless steel cylinders volume cores (100 cm^3) were horizontally inserted in the middle of each sampling layer. Bulk densities were also determined for the topsoil layers (0-5 cm) in the other three MZ. Collected samples were oven dried at 105°C for 48 hours. Average core weights were recorded and used to obtain mean bulk density values per soil layer. For the SOC stocks calculation in the layers below 5 cm in W, F and H management zones, bulk density values obtained from the pit's deeper layers in IZ were used.

For SOC stocks calculations in the entire soil profile in each MZ (0-70 cm) a soil mass correction step was introduced to reduce potential confounding effects due to LUC or management practices. For this, the site with the lowest total soil mass in the IZ was considered, OP-27 years, and all other cores were adjusted to this reference soil mass (Poeplau and Don 2013). Since SOC stocks were calculated for each MZ, calculations were performed by using the corresponding C content, bulk density and layer thickness of each MZ in each OP site. To upscale at the plot level, the proportion that each MZ occupies at the plot level was considered. Therefore, a weighted average mean considering the surface occupied by the four MZs: W=12%; IZ 38%; F=11% and H=39% was used.

To quantify the contribution of OP-derived C and savanna-derived C to SOC stocks, changes in soil δ^{13}C signatures were used in a two sources linear isotopic mixing model (Balesdent et al. 1987):

$$f = (\delta\text{sam} - \delta\text{ref})/(\delta\text{op} - \delta\text{ref})$$

where *f* is the relative proportion of OP-derived C in SOC stocks. δsam is the δ^{13}C of the soil sample at a given layer, δref is the δ^{13}C of the corresponding soil depth from savanna reference soil (C4) and δop is the δ^{13}C of nine fine root biomass C samples (Rüegg et al. 2019). Relative proportions of OP-derived C and savanna-derived C were also calculated for each MZ.

3.3.6 Data analysis

The study was laid out in a randomized split-plot design, with management type as subplot factor (four MZs per main-plot), and study site as the main-plot factor (four OP plots and two savannas). All statistical tests were carried out with RStudio statistical software version 3.4.0 (R Core team 2017). General linear models were used to quantify the effects of management type and time after savanna conversion on all measured biogeochemical variables. Simple linear regressions were performed to test the individual effect of time after savanna conversion (disregarding management type) on bulk SOC stocks, OP-derived and savanna-derived SOC. For this, the linear model "lm" function was used.

Analysis of covariance (ANCOVA) tested the individual and combined effects of management type and time after savanna conversion on all measured biogeochemical variables and SOC stocks by using nested linear mixed-models. These models allowed to represent the study design condition of having four management types nested within each of the sampled OP plots. Such nested structure in the sampling design was included in the random effects part of the model with management type (categorical explanatory variable with four levels: W, F, IZ and H management zones) nested within study sites (categorical explanatory variable with six levels: four OP plots and two savannas). Management type and time after savanna conversion (quantitative variable) were fixed factors. When the interaction between management type and time after savanna conversion (fixed effects) was not significant, the additive model (management type + time after savanna conversion) was used, but if the interaction between the fixed effects was significant then the within model that tested for the effect of time after savanna conversion within management type was used. The linear mixed effect "lme" function of the nlme package was employed to fit the models. Models residuals were assessed to ensure normal distribution and others assumptions of normality and homogeneity of variance. If linear model assumptions were violated, then permutation analyses were conducted for the selected linear mixed-effects model. When the within models were used, the "lstrends" functions in LSMEANS package was used to test for the significance of the slopes of each management type, where significance was declared using 95% confidence intervals (CI 95%) when the intervals did not overlap with zero. Slopes of management types, were further assessed using lsmeans pairwise comparison t-test derived from lme. For all statistical tests, significance was declared at $P < 0.05$ and were evaluated using ANOVA type II sum of squares (car package). If not specified, all discussed differences are significant at $P < 0.05$.

A multivariate approach was used with a redundancy analysis (RDA) and Monte Carlo permutation test (999 permutations) to determine relationships between soil biogeochemical properties and explanatory variables (time after savanna conversion and MZs). RDA was performed using the "rda" function. Canonical variation partitioning with the "varpart" function was performed to assess the individual and collective contributions of time after savanna conversion and management type to the total variation in soil biogeochemical variables.

3.4 Results

3.4.1 Ecosystem C stocks

Savanna conversion into OP plantations increased total ecosystem C (TEC) stocks (AGB, BGB and SOC). Even though OP plantations develop large biomass, the soil was the major C pool holding between 82% in the 8-year old and 62% in the 27-year old plantations of the total C in OP agroecosystems (Fig. 1). Total OP biomass (AGB + BGB) increased linearly with cultivation time at a rate of 2.8 ± 0.17 Mg C ha^{-1} yr^{-1}, corresponding to a time-averaged OP biomass C stocks of 42.2 ± 0.17 Mg C ha^{-1}. Such time-averaged OP biomass C stocks was about twenty times higher compared to savanna total biomass C stock, 2.1 ± 0.11 Mg C ha^{-1}. Thereby, the increase in OP TEC stocks compared to savanna can be attributed mainly to the striking increase in total OP biomass with time.

Time-averaged TEC stocks down to 70 cm depth were of 164.1 ± 9.6 Mg C ha^{-1} and 124.0 ± 9.3 Mg C ha^{-1} in OP plantations and savannas, respectively, thus savanna conversion into OP plantations implied a positive ecosystem C balance with a net gain of 40.1 ± 13.4 Mg C ha^{-1} (Fig. 1). Thus, the estimated C sequestration potential of OP cultivation on former savanna areas in the Altillanura region of Colombia would be of 147.2 Mg CO_2 eq. ha^{-1}.

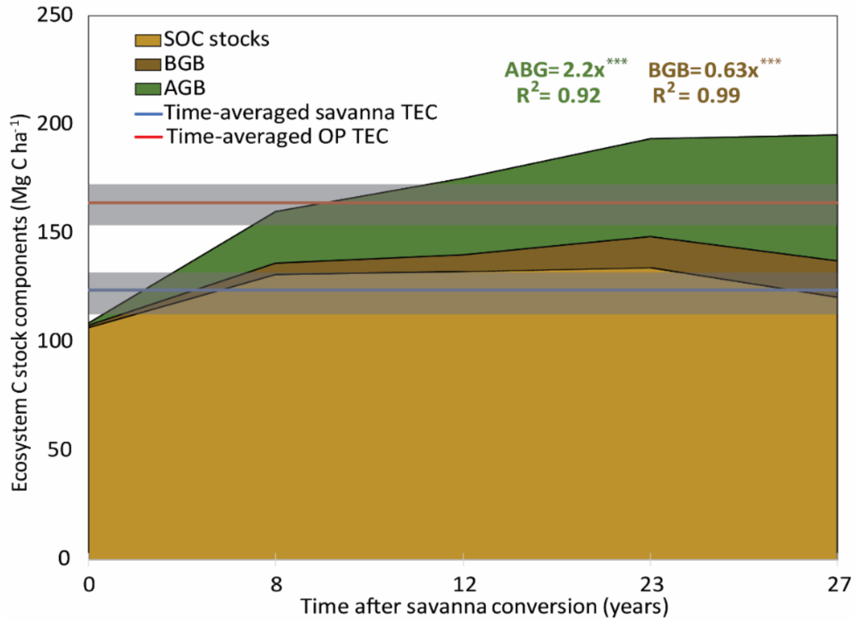

Figure 1. Ecosystem C stocks components in OP plantations and time-averaged TEC stocks in OP plantations (orange line) and savannas (blue line) over an OP-rotation cycle, including the soil to 70 cm depth. The shaded gray areas represent SE. SOC accumulation rates (Mg C ha^{-1} yr^{-1}) in the whole soil profile, ABG and BGB are indicated for the entire plantation cycle. AGB, aboveground biomass; BGB, belowground biomass and SOC, soil organic carbon stocks.

3.4.2 Soil C stocks dynamics

Bringing former savanna areas into OP cultivation did not alter SOC stocks at the plantation scale after 27 years in the soil profile down to 70 cm. Mean SOC stocks across OP sites and savannas was of 121.9 ± 9.3 Mg C ha^{-1} (Fig. 1a). However, application of external inputs either as mineral fertilizers or as crop residues, led to increases in SOC stocks by 1.9 ± 0.3 Mg ha^{-1} yr^{-1} and 1.8 ± 0.6 Mg ha^{-1} yr^{-1} in areas where high fertilizers and root inputs (W) and large crop’s residues inputs form pruned OP fronds (F) occurred, respectively. By contrast, SOC stocks remained constant at areas with low- (IZ) and no-inputs (H) (Fig. 2a, Table 1). Because the small share that the W and F areas represent within a plantation (23% together), the overall SOC stocks did not increase (Fig. 1). In W and F, this SOC dynamic over time was very similar in each of the three uppermost surface soil layers (0-5, 5-10 and 10-

20 cm) (Table S1). At the three deepest soil layers (20-30, 30-50 cm and 50-70 cm depths) changes in SOC stocks due to savanna conversion or OP management practices were not detected (Table S1).

Management type also affected the dynamics of both OP- and savanna-derived C with cultivation time. In the entire soil profile, the accumulation of OP-derived C under high fertilizers (W) and large crop residues (F) inputs followed the same pattern as the bulk OP-derived SOC stocks at these same areas (Fig. 2b). In spite of the absence of detectable significant change in bulk SOC at the low- (IZ) and no-inputs (H) areas, OP-derived C increased slightly in these MZs at similar rates, substituting savanna-derived SOC. New OP-derived C accumulation rates were of 2.4 ± 0.3 , 2.1 ± 0.3, 0.8 ± 0.2 and 0.6 ± 0.2 Mg ha^{-1} yr^{-1} in W, F, IZ and H, respectively (Table 1). Total SOC consisted predominantly of savanna-derived C across all the study time. The proportion of OP-derived C in the total SOC pool across management zones, considering their relative contribution to the surface in the plot was very modest and represented about 0, 8.6, 15.1 and 23.3% after 8-, 12-,23- and 27-years of OP cultivation, respectively.

At the large crop residue (F) and high fertilizers (W) input areas, the proportion of new OP-derived C in the full profile was as high as 36 and 45 %, respectively, after 27-years of OP cultivation (Fig. 2c). The uppermost soil layer (0-5 cm) changed from predominantly savanna-derived C to OP-derived C after 27-years of OP cultivation at high input areas (W and F), but at low- and no-input areas nearly 50% of the total C was still of savanna origin (Table S2). At the four subsequent soil layers (5-10; 10-20; 20-30 and 30-50 cm), OP-derived SOC stocks increased importantly under F and W with time, but no or minor changes were detected at the IZ and H areas. Interestingly, in the deepest soil layer (50-70 cm), new OP-derived C increased with time at all four MZs (Table S3), but this had no effect on the bulk SOC stocks. At this same layer, the OP-derived C accumulation rate in the high fertilizer input area was the highest, 0.13 ± 0.01, followed by the large crop residue input area, 0.08 ± 0.02. On the other hand, savanna-derived C stocks decreased in the three uppermost soil layers but decomposition rates were not significantly affected by MZs (Fig. 2d, Table S3).

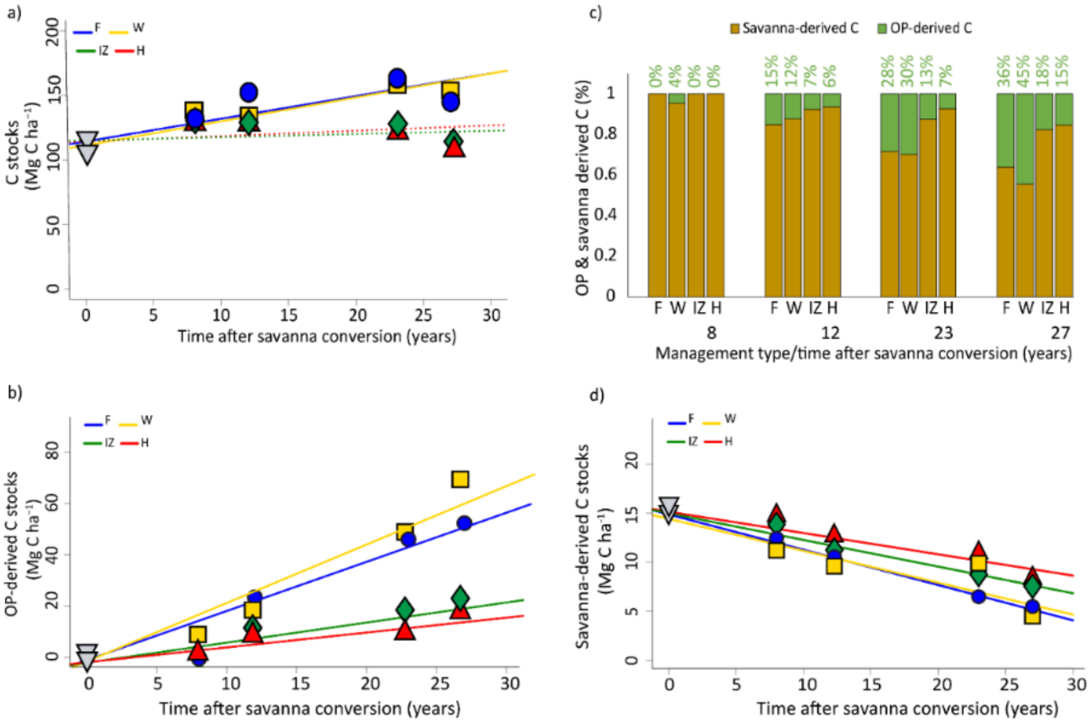

Figure 2. Soil C dynamics in oil palm plantations established on former savanna areas from. a) Soil C stocks for each management type over time in the full profile (0-70 cm); b) Oil palm-derived C accumulation according to management type over time in the full profile; c) Proportion of oil palm-derived (numbers on top of the bars) and savanna-derived C for each management type over time in the full profile and d) Savanna-derived C dynamics at each management type in the topsoil (0-5 cm). Management types are: F, frond pile; W, weeded circle; IZ, interrow and H, harvest path. Solid lines represent significant changes with time.

Table 1. Bulk C accumulation rates, savanna- and OP-derived SOC stocks at the weeded circle (W), frond piles (F), interzone (IZ) and harvest path (H) management zones in the entire soil profile (0-70 cm).

Management zone	Proportion of the total plantation area (%)	Accumulation rates (Mg ha^{-1} yr^{-1})	Savanna-derived C stock (Mg C ha^{-1})	OP-derived accumulation rates (Mg ha^{-1} yr^{-1})
W	12	1.9 (0.3)	55.4	2.4 (0.3)
F	11	1.8 (0.6)	64.0	2.1 (0.3)
IZ	38	NS	82.3	0.8 (0.2)
H	39	NS	84.8	0.6 (0.2)

NS: not significant

3.4.3 Soil chemical and biological fertility

Overall, the regular application of fertilizers and crop residues led to a positive effect on soil surface (0-5, 5-10, 10-20 and 20-30 cm layers) nutrient dynamics (Table S4). Sum of cations (Ca, Mg, K, Na) values were the greatest in areas receiving high fertilizer and large crop residues inputs at all soil layers. In the top 5 cm soil layer, these two inputs-receiving areas contained about 20 times more base cations than native savannas after 27-years of OP cultivation (Fig. 3a). At the other two areas with low- and no-inputs applications, changes in exchangeable cations in the top 5 cm were not detected over time.

However, at all the four layers below 10 cm, except at the 30-50 cm layer, the sum of cations values increased only due to time after savanna conversion and not in relation to management type (Table S4).

Not only major cations, but also available P increased with OP cultivation time at areas receiving high fertilizer inputs (Fig. 3b). However, this increase in available P was not observed at F. The available P improvement occurred at all depths except at the two deepest soil layers (30-70 cm), where no change in available P content was detected (Table S4). Similar to the dynamics of exchangeable cations, the low- and no input areas did not vary in available P content between management types at any soil layer. Soil C content increased in a similar fashion at the three uppermost soil layers over time at areas with high fertilizer and large crop residues inputs. At areas with low- and no-inputs, changes over time were not detected. Also, differences in C content between management types increased with time after savanna conversion. Topsoil C content ranged from 2.1 to 5.5% across all management types and natural savannas. At areas with large crop residues and high fertilizer inputs, soil C content peaked at 23-years after savanna conversion by about three- and two-fold, respectively (Fig. 3c).

There was no effect of time and management type on soil pH in the three uppermost soil layers (0-5, 5-10 and 10-20 cm layers). However, in the area subjected to high fertilizer input there was an acidification at the 30-50 cm soil layer (Table S4). Management practices altered isotopic N dynamics. Unexpectedly, soil δ^{15}N signatures decreased progressively with OP cultivation time at areas with large crop residues inputs in the 0-5 and 5-10 cm soil layers (Fig. 3d). Although not significant, at areas subjected to high fertilizer input there was also a declining trend as in areas with large crop residues inputs (Table S4).

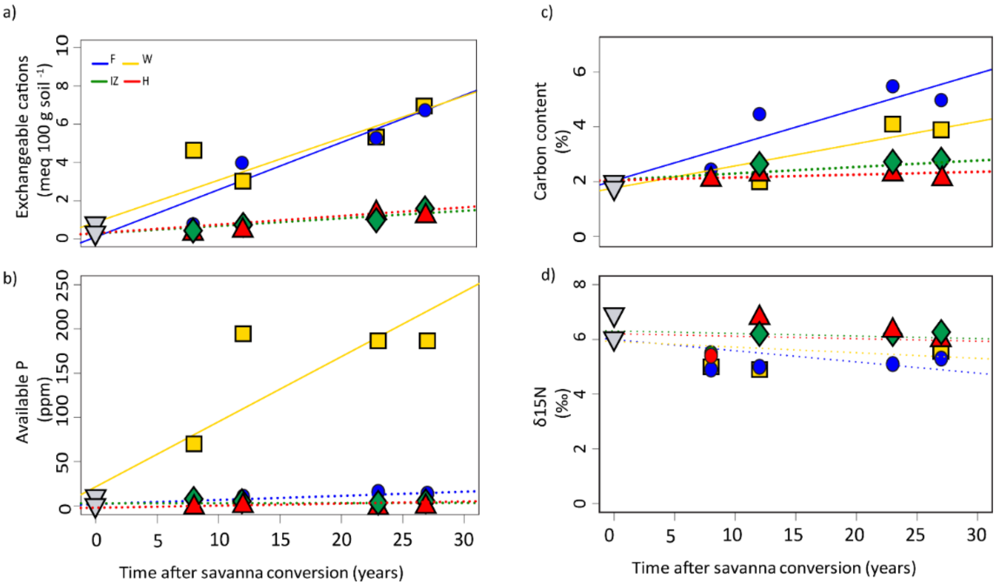

Figure 3. Soil nutrient dynamics in oil palm plantations established on former savanna areas in the 0-5 cm layer. a) Sum of cations; b) Available Bray-P; c) Soil C content; d) N signature. Management types are: F, frond pile; W, weeded circle; IZ, interrow and H, harvest path. Solid lines represent significant changes with time.

In general, the majority of the soil microbial traits were influenced either by management type, time after savanna conversion or both in the topsoil (0-5 cm; Table 2) and few or no effects were detected in the subsoil (Data not shown). The soil surface of areas where crop residues were applied (F), were the most biologically active. There, microbial respiration, NH_4 production and enzymatic activities of leucine had the highest values. Microbial respiration also increased with OP cultivation time at the surface of soils with high fertilizer and crop residues inputs (Table 2). While soil MBC decreased at areas with low- (IZ) and high fertilizer inputs (W), no changes were detected at areas with high crop residues inputs (F) (Table 2). Irrespective of management type, the microbial and metabolic quotients decreased and increased, respectively, with time after savanna conversion.

Table 2. Temporal rate of changes and SE in soil biological variables in the topsoil (0-5 cm) over one OP rotation cycle. Results of ANCOVA linear mixed-models. *P*-values are indicated with the respective symbols *** for *P*<0.001, ** for *P*<0.01, * for *P*<0.05 and NS for *P*>0.05.

Soil properties	Unit	Time effect	Management effect	Management/time	Management practice		
					W	IZ	F
Microbial respiration	mg C kg^{-1} soil day^{-1}	NA	NS	***	0.84 (0.14)**	NS	1.30 (0.29)*
Microbial biomass C	mg kg^{-1}	NA	NS	***	-6.79 (2.3)*	-8.07 (1.59)**	NS
Ammonium	mg kg^{-1}	NA	NS	***	NS	NS	5.02 (0.93)**
Leucine aminopeptidase	nM MUC $g^{-1}h^{-1}$	NA	NS	***	NS	-0.98 (0.54)*	2.11 (0.78)*
Microbial quotient	NA	-0.41 (0.05)***	NS	NA	NA	NA	NA
Metabolic quotient	NA	0.009 (0.002)***	NS	NA	NA	NA	NA
DOC	µg C g^{-1}	NS	*	NA	NA	NA	NA
Phosphatase	nM MUF $g^{-1}h^{-1}$	NS	*	NA	NA	NA	NA

DOC: dissolved organic carbon (K_2SO_4-extract); NA: not applicable. Values are rates of change over one OP cultivation cycle (27-years) with standard errors in parentheses. When the interaction between time after conversion and management type was significant the model with the effect of time after savanna conversion within management type was used.

The relative role of time after savanna conversion and management practices (W, F and IZ) on soil biogeochemical properties were quantified and synthesized in a multivariate analysis. RDA showed that regardless of time after savanna conversion into OP, soils of each MZ cluster together, forming three well defined groups characterized by specific biogeochemical properties (Fig. 4). Both axis 1 and 2 were statistically significant and accounted for 32.8% and 22.7% of the total variation in soil biogeochemical properties, respectively. Overall, areas with high fertilizers (W) and large crop residues (F) inputs, favored either high soil chemical fertility or high soil biological activity. More specifically, the area with large crop residue inputs had the highest soil biological activity, *i.e.* MBC, microbial respiration and potential enzymes activity, and the area with high fertilizer inputs had the highest soil chemical fertility, *i.e.* sum of cations, P and NO_3 (Fig. 4). Management type explained much more variation (44%) in soil biogeochemical properties than time after savanna conversion (9%).

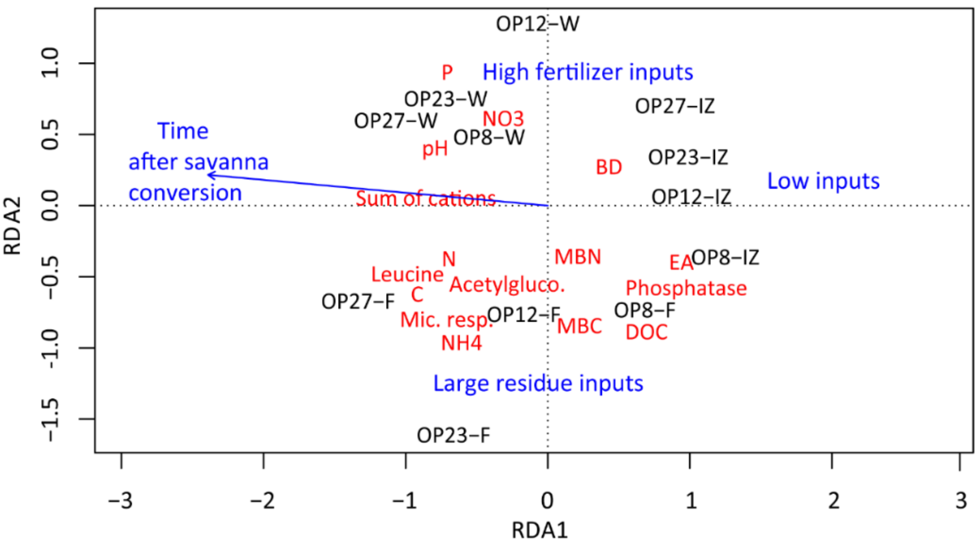

Figure 4. Redundancy analysis (RDA) of soil biogeochemical variables in the topsoil (0-5 cm) constrained for time after savanna conversion and management zones. P, available phosphorus (Bray P); NO_3, nitrate; NH_4, ammonium; BD, bulk density; MBN, microbial biomass N; MBC, microbial biomass C; EA, exchangeable acidity; DOC, dissolve organic C; Mic. resp, microbial respiration. The OP plots are indicated as OP##-X, where ## is the years after savanna conversion and X is the management zone; large residue inputs (F), high fertilizer inputs (W) and low inputs (H).

3.5 Discussion

The prevailing notion that converting natural ecosystems into cultivated land always negatively affects ecosystem C stocks does not hold in the case of savanna conversion into OP. Our study is the first to present evidence of the impacts of tropical natural savannas conversion into OP across a full cultivation cycle on soil biogeochemical properties down to 70- cm and ecosystem C stocks. According to the first hypothesis, savanna conversion into OP resulted in a positive ecosystem C balance (40.1 Mg C ha^{-1}), with potential to be further enhanced if better soil management practices are adopted. This contrasts with other LUC types, in particular with the deforestation scenario where it has been shown that OP plantations expansion on forested areas led to striking ecosystem C losses, amounting to 173 Mg C ha^{-1} (Guillaume et al. 2018). However, while deforestation usually results in large TEC losses (total biomass and SOC), other deforestation free alternatives like pasture conversion into OP result in trade-offs between major TEC components, where gains in AGB are offset by severe losses of pasture-derived C, which renders this alternative LUC neutral in terms of ecosystem C balance (Quezada et al. 2019) (Fig. 5). Such, SOC loss did not occur in this study, because the decomposition of savanna-derived C was minimal with cultivation time and was offset by OP-derived C at all soil layers (Table S3). Consequently, in the conversion of fine-textured savanna areas into OP, the gains in C biomass are not offset by SOC losses and this led to a net positive ecosystem C outcome for this important ongoing LUC type in Colombia. The values found for AGB and BGB stocks, rates of change and time-averaged stocks in OP and savanna are similar to previously published results (Guillaume et al. 2018; Kotowska et al. 2015; Ramírez-Restrepo, Vera, and Rao 2019; Ziegler et al. 2012).

Our findings contrast with those in Brazil where lower SOC stocks were reported in OP plantations of pasture origin after 8 years of cultivation (Frazão et al. 2013). Similarly, in another study conducted in the same savanna region of Los Llanos, reduced SOC stocks in a 9-year old plantation with sandy soils were found (Rüegg et al. 2019). Unlike to the two above mentioned studies, in grassland derived OP plantations increased bulk SOC stocks and no losses of grassland derived C were reported (Goodrick, P. N. Nelson, et al. 2015). These authors suggested that the presence of black C produced during grassfires explained partly the high persistence of the original grassland C in OP plantations. In our study, the relatively slow savanna-C decomposition over time could be explain by physical stabilization of old savanna C due to high content of minerals that protected SOC from mineralization, *i.e.* clay, iron and aluminum oxides and hydroxides, as suggested in other studies in tropical areas (Baldock and Skjemstad 2000; Barthès et al. 2008). The pasture-derived plantations of the same region that experienced SOC losses had lower clay content (Quezada et al. 2019).

Notably, our results demonstrate that the adoption of soil management practices that nourish the soil with mineral nutrients and OM can increase the potential of savanna conversion as an important long-term C sink. However, this can be possible only if the benefits of increased SOC stocks, enhanced fertility and soil microbial activity observed under the W and F areas can be scaled-up to the whole plantation level. In partial support to our second hypothesis, not only the application of crop residues in F, but also chemical fertilizers in W, led to SOC accumulation rates in the whole soil profile, between 1.8 and 1.9 Mg C ha^{-1} yr^{-1}. Such accumulation rates were similar to the one found for increased AGB in OP plantations in one full rotation cycle, 2.2 Mg C ha^{-1} yr^{-1} (Fig. 1). It is noteworthy that the surface receiving high chemical fertilizers close to the palm trunk (W) is also subjected to steadily increasing inputs of root biomass and the surface receiving large crop residues (F) is subjected to some fertilizer inputs and to belowground inputs via OP root biomass when plantations are older than 5-6 years (Rüegg et al. 2019).

The accumulation of bulk SOC stocks was prompted by substantial increases in OP-derived C that largely surpassed the decomposition of savanna derived-C in those zones (Table S3). As found in other studies (Carron et al. 2015; Frazão et al. 2013; Rüegg et al. 2019), areas receiving high fertilizer inputs (W) had the highest SOC stocks. The SOC increases we observed at W could have been facilitated by the high nutrient supply and availability that stimulated fine root growth and, so greater belowground OM inputs (Rüegg et al. 2019). This is of relevance, because C storage in soils is less vulnerable to losses compared to biomass C storage. Thereby, adequate management of external inputs have an important role in enhancing the C storage capacity of OP agroecosystems, so that the C benefits of savanna conversion could result not only from increased biomass C, but also from a simultaneous gain in SOC stocks, which could eventually double the C sequestration potential of savanna conversion.

Increases in bulk SOC stocks due to either organic or chemical inputs were limited to the three uppermost soil layers. This compares to a recent global meta-analysis, where the effects of cover crops were limited to the top 30 cm (Poeplau and Don 2015), which could be explained by reduced C and nutrient inputs into subsoil layers. In addition, the Intergovernmental Panel on Climate Change (IPCC) considers a twenty year timeframe as the period required to reach a new SOC equilibrium (Chenu et al. 2019). Here, we did not see any sign of SOC stocks approaching a new steady state in the top 20 cm when inputs where applied, which also contrasts with other studies where it has been shown that SOC sequestration capacity becomes smaller with time after LUC or the implementation of better management practices like reduce tillage (Sommer and Bossio 2014; West and Six 2007). Furthermore, from the savanna-derived C decomposition rates, we did not observe that nutrients or crop residues inputs increased old savanna C mineralization as a result of priming effect at any soil layer (Table S3).

In contrast to the similar rates of C accumulation observed at input receiving areas W and F, SOC stocks remained unchanged with cultivation time in areas where reduced or almost no above- and belowground inputs occurred (IZ and H), despite IZ received nutrients during the fertilization process (Table S1). All these factors together could indicate that both OM and nutrients inputs benefited synergistically soil C accumulation at the W and F zones. This is in agreement with the findings in a mature plantation in the same savanna area where it was demonstrated that the positive effects of nutrients on SOC accumulation were driven by C inputs via OP roots (Rüegg et al. 2019). Moreover, inputs additions in our study resulted in accumulation rates greater to those reported in long-term and meta-analysis studies where conservation agriculture practices like reduced tillage, residues retention, use of cover crops and others were implemented (Batlle-Bayer et al. 2010; Lal, Negassa, and Lorenz 2015; Mazzoncini et al. 2011; Tautges et al. 2019).

The third hypothesis was only partly confirmed since areas of high fertilizers (W) and those of crop residues (F) inputs differed in their soil chemical and biological properties. We found that SOC pools, such as microbial C, decreased at W and remained unchanged at F, but potentially mineralizable C, measured by soil respiration, and enzymes activity were higher at F than at W and savannas. In general, adding crop residues at F supported a level of biological activity similar to that in natural savannas. This is an important perspective, because it is not very common that managed agroecosystem can maintain similar levels of soil biological activity compared to its natural counterparts. Although we do not have direct evidence on the distribution of C between SOC fractions, we believe that the distribution of total SOC varies among the various C pools so that at W most of the new C was accumulated at more stable C pools, whereas the greatest microbial activity (enzymes and microbial respiration) in the aboveground OM inputs zone (F) increased the labile C pools with fast turnover rates. This reflects the importance of further analyses of the soils used in this study with more advanced techniques, *i.e.* SOC fractionation and isotopic tracer, to gain a thorough mechanistic understanding of SOC stabilization.

The application of large amounts of fertilizers in W resulted in reduced soil microbial activity, but increased soil chemical fertility compared to the application of crop residues at F. Very likely the overall soil conditions at F favored better soil biological activity compared to W. This highlights trade-offs between agronomical management purely based on fertilizers addition and soil biological functioning. Further, it has been demonstrated that the accumulation of OP fronds on the soil surface is not an efficient practice for SOC stabilization and that the positive effects on SOC stocks are mainly from the improved soil conditions under the decomposing fronds like nutrients availability and moisture (Rüegg et al. 2019). Thereby, we can draw similar conclusions because if accumulation of OP fronds (F) would be efficient, then the accumulation rates of SOC would have been much higher at F than at the area close to the trunk (W). So far, there is limited knowledge on the response of soil C cycling to nutrient enrichment (Soong et al. 2018), but in OP plantations it has been shown that nutrient imbalances result from excessive fertilizers application (Carron et al. 2016), which in turn jeopardizes long-term crop productivity and sustainability.

With all the changes in soil physico-chemical and biological characteristics discussed before, it is possible that the structure of soil microbial communities varied between MZs, *i.e.* bacterial:fungal ratios. The increase with time after savanna conversion of the metabolic quotient, at all MZs, could be indicative of a shift in soil microbial community structure to bacteria-based food web channels and r-strategists, characterized by lower C use efficiency and faster turnover rates compared to fungal-based food webs. To date, studies on soil microbial communities in OP have used composite samples from all MZs, therefore virtually no data exist on soil microbial populations at each MZ. In the typical deforestation LUC to OP, no clear patterns have been reported, however it has been suggested that OP plantations have greater soil biota functional diversity than forest due to the high heterogeneity of resources within plantations, *i.e.* nutrients, above-, belowground inputs (Tripathi et al. 2016). Some studies have reported no changes in the overall soil bacterial diversity (Lee-Cruz et al. 2013), decreases in fungal diversity (Kerfahi et al. 2014) and declines and increases in certain bacteria groups and arbuscular mycorrhizal fungi with deforestation to OP (Krashevska et al. 2015; Tripathi et al. 2016). This insufficient characterization of soil microbial communities within OP plantations underscore the need for further research in soil microbial related aspects.

The relative importance of management practices and LUC to explain the variation on soil biogeochemical properties is a key finding of this study. Management had about five times more importance in explaining soil biogeochemical variations than LUC from savanna to OP, meaning that the magnitude and direction of changes in soil biogeochemical properties are much more related to the cultivation models than the LUC type performed. This highlights that OP production systems can tackle a more sustainable expansion, if the adopted LUC options and management strategies achieve a better compromise between environmental and agricultural production goals. In the management strategy scenario, we propose a more ecologically oriented intensification approach. Although there is so far little perspective, recent on-farm experimental results have shown that development of understory vegetation in OP plantations can improve various environmental aspects without affecting productivity (Ashton-Butt et al. 2018; D. 2019). We propose that instead of allowing a total cover of the OP plantations' understory surface area, the regrowth of natural vegetation should be focused on the IZ areas. This recovery of natural vegetation within plantations can serve as a buffer against biodiversity decline and thereby increase beneficial insects and mammal's species, with potential benefits for pest and disease control and thus reduction of pesticides applications. In addition, soil conditions could also be enhanced by increasing soil biodiversity and therefore soil nutrient cycling and SOC stocks.

Given the inherent low nutrient contents of savanna soils and tropical soils in general, the supply of OM should go hand in hand with a balanced supply of mineral nutrients via fertilizers, so that substrate stoichiometry does not constrain soil microbe's growth and activity (Kirkby et al. 2013). Additionally, replacing partly the large synthetic fertilizers amounts typically used in commercial OP plantations by organic nutrient sources like compost from OP mill waste, can reduce greenhouse gas emissions, *i.e.* N_2O emissions, and increase soil aggregation (Mpeketula and Snapp 2019; Tautges et al. 2019). Although we recognize that a reduced-input biological strategy can be challenging at the field-scale, the environmental benefits that might arise from it are various. Such benefits can include increased C sequestration, promotion of beneficial organisms like pollinators, natural enemies and soil biota, soil aggregation and soil biogeochemical functioning at the whole surface area of OP plantations without affecting OP productivity and possibly increasing or maintaining its profitability (Rochmyaningsih et al. 2019).

Under the LUC scenarios options, here we showed empirically that savanna conversion into OP can effectively result in a positive ecosystem C budget. This beneficial ecosystem C outcome is highly relevant in the context of reducing C emissions from deforestation and the ongoing expansion of OP on savanna areas. However, it is important to note caveats on the expansion of agriculture in savanna regions. Recent studies suggest both, low and high impacts on savanna biodiversity due to its conversion into OP plantations in Los Llanos region of Colombia (López-Ricaurte et al. 2017; Ocampo-Peñuela et al. 2018; Prescott et al. 2016). In this unclear context, future expansion is not recommended on savanna unique ecosystems. Instead, expansion should happen preferably in previously transformed land like degraded pastures areas that could be converted into OP to minimize the impacts on biodiversity and avoid the large ecosystem C losses and other environmental burdens of deforestation (Gilroy et al. 2015; Quezada et al. 2019). Taken together, our results and recommendations for future OP cultivation emphasize the perspective of its expansion on previously cultivated land, *i.e.* pasture areas, and the need for an integrated reduced-input management approach where insights of natural and agricultural sciences coincide in a practical scheme to enhance the functioning of agroecosystems. Finally, while we provide here some elements for a more sustainable OP agriculture, further research is warranted to quantify the complex interplay and outcomes that might exist between the recommended practices and the discussed deforestation-free LUC alternatives on other important environmental aspects.

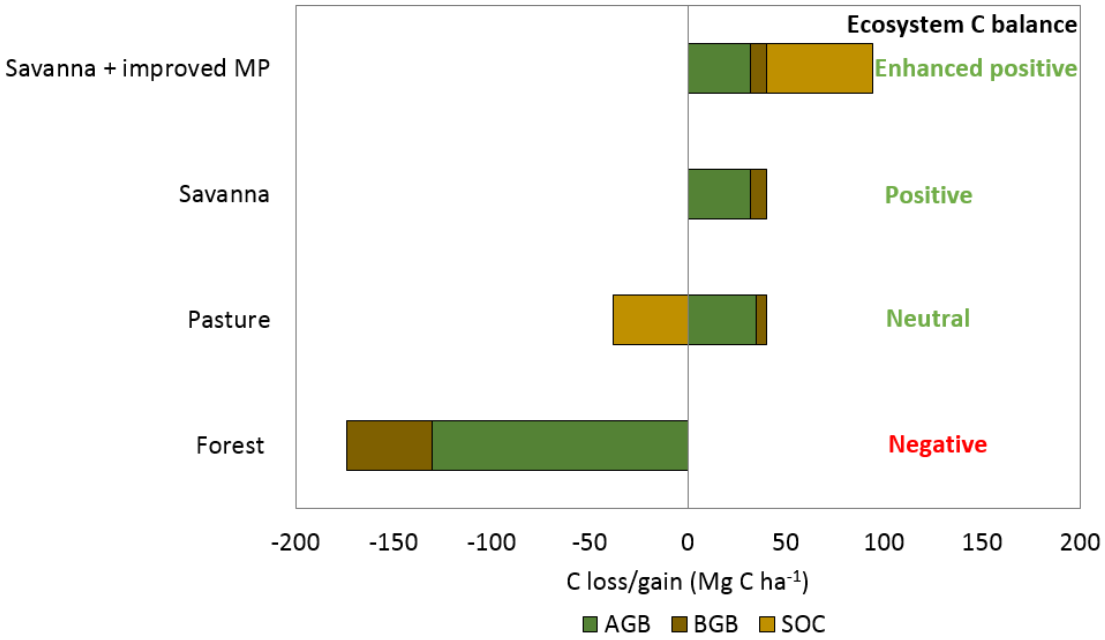

Figure 5. Main ecosystem C component changes in land use change to OP. Pastures are derived from savannas and then converted into OP. Savanna + improved management practice (MP): increased in SOC stocks assuming that both C and nutrients are added according to the proposed reduced-input management strategy. Data origin is as follow: forest to OP (Guillaume et al. 2018); pasture to OP (Quezada et al. 2019); savanna to OP and savanna + improved MP are from this study.

4 Manuscript 3: Drivers of soil carbon stabilization in oil palm plantations

“I have not ***failed****. I've just found 10,000* ***ways*** *that* ***won't work****”*

Thomas A. Edison

Oil palm plantation in the savanna region of Los Llanos in Colombia. On the bottom, field work in savanna areas.

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4.1 Abstract

Increasing soil organic carbon (SOC) in agroecosystems is necessary to mitigate climate change and soil degradation. Management practices designed to reach this goal call for a deeper understanding of the processes and drivers of soil carbon input stabilization. We identified main drivers of SOC stabilization in oil palm plantations using the well-defined spatial patterns of nutrients and litter application resulting from the usual management scheme. The stabilization of oil palm-derived SOC (OP-SOC) was quantified by δ^{13}C from a shift of C4 (savanna) to C3 (oil palm) vegetations. Soil organic carbon stocks under frond piles were 20 and 22 % higher compared to harvest paths and interzones, respectively. Fertilization and frond stacking did not influence the decomposition of savanna-derived SOC. Depending on management zones, net OP-SOC stabilization equalled 16-27% of the fine root biomass accumulated for 9 years. This fraction was similar between frond piles and litter-free interzones, where mineral NPK fertilization is identical, indicating that carbon inputs from dead fronds did not stabilize in SOC. A path analysis confirmed that the OP-SOC distribution was largely explained by the distribution of oil palm fine roots, which itself depended on management practices. SOC mineralization was proportional to SOC content and was independent on phosphorus availability. We conclude that SOC stabilization was driven by C inputs from fine roots and was independent of alteration of SOC mineralization due to management. Practices favouring root growth of oil palms would increase carbon sequestration in soils without necessarily relying on the limited supply of organic residues.

Keywords: carbon isotopes - fertilization - fine roots - microbial activity - structural equation modelling - savanna – Colombia

4.2 Introduction

Soil organic carbon (SOC) depletion in agroecosystems is a major source of greenhouse gas emissions, resulting in losses of soil fertility and ecosystem stability (Amundson et al. 2015). Policymakers have recently acknowledged the promotion of soil C sequestration in agroecosystems as a promising strategy to simultaneously mitigate climate change and enhance food security (Lal 2016). Nonetheless, management practices favouring soil C sequestration lead to highly variable outcomes, calling for a deeper understanding of processes and factors controlling C stabilization, especially after land-use changes (Ghimire et al. 2017; Haddaway et al. 2017). This is particularly needed in regions that are undergoing rapid and substantial land degradation following conversion from largely forested landscapes to intensive agricultural systems, as has been the case in tropical landscapes dominated by oil palm plantations. In Sumatra, a region with a long history of oil palm cultivation, a significant proportion of oil palm plantations has already reached a critical low level of SOC content (< 1%) in the topsoil (Guillaume, Holtkamp, et al. 2016).

Stocks of SOC depend on the balance between soil C inputs from vegetation and outputs from SOC mineralization, erosion and leaching (Lorenz and Lal 2018). This balance is strongly affected by the conversion of natural ecosystems to intensive agricultural land (Guillaume et al. 2018). Predicting impacts of land-use change and management on SOC dynamics and its stabilization faces major difficulties as many factors affect both litter input and mineralization processes that determine the fraction of C input stabilized in SOC. While higher plant biomass inputs might lead to higher SOC accumulation, an increase of fresh organic matter inputs may enhance the mineralization of more recalcitrant SOC that ultimately reduces the gain in SOC, a process known as priming (Kuzyakov, Friedel, and Stahr 2000). Nutrient application modifies the stoichiometry of organic matter inputs and of soil organic matter, which in turn affects microbial processes controlling SOC stabilization (Qiao et al. 2016; Zang, Wang, and Kuzyakov 2016). For instance, altered microbial carbon use efficiency (i.e. the ratio of C incorporated into microbial biomass to the added C) or mining for nutrients from recalcitrant SOC result in either SOC gains or losses (Finn et al. 2016; Kirkby et al. 2014). Quantifying soil C inputs remains a methodological challenge and few data are available, for instance, on roots turnover, rhizodeposition, and the fraction of aboveground litter C stabilized in SOC (Pausch and Kuzyakov 2018). Experimental data are especially limited for perennial plants in tropics and subtropics.

The fast SOC turnover in the tropics makes soils particularly sensitive to land-use change (Guillaume et al. 2015; Pabst et al. 2016; Zech et al. 1997). For example, soil C inputs decrease up to 90% when rainforests are converted to oil palm plantations, resulting in a rapid drop of SOC (Guillaume et al. 2018). Soil organic C losses are not, however, uniform within plantations, and specific management zones within the plantation may even exhibit a gain in SOC (Khasanah et al. 2015; Rahman et al. 2018a). Soil C inputs in mature oil palm plantations without cover crops (the most common practice) occurs mostly belowground through rhizodeposition because the understory is frequently cleared. Significant aboveground C inputs occur only under frond piles, i.e. zones where dead fronds are stacked. A gradient of fertilizer application is superimposed on the gradient of soil C input: most fertilizers are applied around the trunks and, in some cases, additionally to the whole surface area at lower rate, including the frond piles but excluding the harvest path. These management practises lead to characteristic management zones with specific factors affecting SOC dynamics (Fig 1a). With plantation ageing, the heterogeneity of SOC distribution increases, depending on the distance to the tree, the presence of frond piles and fertilizer applications (Frazão et al. 2013; Goodrick, P. Nelson, et al. 2015). Carbon and nutrients cycling are, thus, highly heterogeneous leading to a variable SOC equilibrium depending on management practices. This highlights the possibility to promote the increase of overall SOC stocks by redesigning management practices to reach this goal.

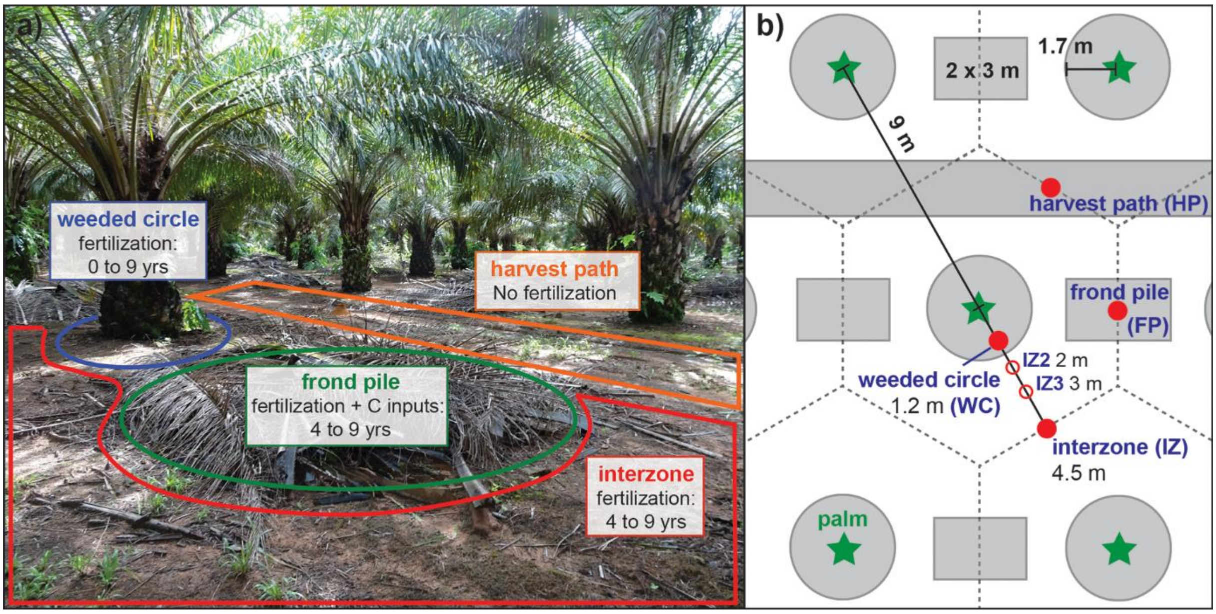


Figure 1. Management zones in a mature oil palm plantation. A, Four management zones varying in terms of fertilization and aboveground C inputs. B, Sampling points (red dots) in the 9-year old plantation. Frond piles and harvest path are absent in the 2- and 4-year old plantations but two additional points (empty red dots) were sampled to assess palm roots lateral extension.

In Colombia, oil palm plantations are often established on native savanna grasslands dominated by grasses with a C4 photosynthesis pathway. The shift from C4 (grasses) to C3 vegetation (oil palm) allows source determination in soil organic matter using its δ^{13}C signature (Balesdent and Mariotti 1987). The aim of the present study is to disentangle the effects of soil C and nutrient inputs on newly accumulated SOC (oil palm-derived) and the decomposition of old SOC (savanna-derived), taking advantage of the specific patterns of fertilization and soil C inputs in the four management zones. We hypothesized that soil C inputs and consequent SOC accumulation increase with fine root density, which itself depends on plantation age and distance to trees, as well as on the presence of frond piles. Fertilizer application is, however, expected to decrease SOC stabilization rates where soil C inputs are low. Hence, the specific aims of the study are to i) quantify the new oil palm-derived (C3 signature) and the old savanna-derived SOC stocks (C4) in a mature oil palm plantation established on native savanna grassland, ii) assess oil palm root development and its impact on SOC accumulation with plantation age, iii) determine the impacts of management practices on root development and soil microbial activity and iv) identify the main factors (root density, nutrient availability, microbial activity) driving SOC stabilization.

4.3 Material and methods

4.3.1 Study area

The study was conducted in the Eastern Plains (*Llanos Orientales*), Department Meta, Colombia (4°05'7.0"N, 71°53'59.0"W). The region experiences a tropical climate (mean annual temperature of 26 °C and yearly precipitation of 2200 mm yr^{-1}) with a distinct dry season from December to March and 95% of the yearly rain falling between April and November (Lavelle et al. 2014a; Rippstein et al. 2001). The study site lies in the slightly undulating well-drained high plains (Altillanura plana) dominated by Plinthosols and Ferralsols (IUSS Working Group WRB 2014). These soils have a low fertility, high acidity and high aluminium saturation limiting agricultural production (Lavelle et al. 2014a; Rippstein et al. 2001). The natural vegetation is an herbaceous savanna with scarce bushes, which is drained by many small rivers. Gallery forests (morichales) grow in the depressions along these rivers (Rippstein et al. 2001).

Two unmanaged native savanna sites and three oil palm plantations with increasing age (2-, 4-, and 9-years old) were selected within an area of approximately 8 × 8 km and sampled in July and August 2016 (Fig. S1). Sites were carefully chosen with the help of plantation agronomists to ensure that the investigated plantations had been established on unmanaged native savannas, i.e. no cattle grazing in the past. Soils in the five selected sites were classified as sandy-loam Ferralsols (clay content and pH ranging from 11 to 16% and from 4.5 to 5.3 in the top 30 cm, respectively) with compacted top soil (bulk density ranging from 1.31 to 1.46 g cm^{-3} in the top 10 cm). Drivers of SOC stabilization were assessed in the 9-year old plantation. The 2- and 4- years old plantations were selected to assess the development of oil palm rooting system and its relationship with SOC accumulation.

4.3.2 Plantation management

Oil palm plantations had been established on native savannas whose soils were first loosened with a chisel plough (to a depth of 40 cm) and tilled with an overturning plough (10 cm). Liming and phosphate rocks were applied before planting palms. Oil palm trees had been planted in a triangular grid pattern with a distance of 9 m between trees, leading to 143 palms ha^{-1} (Fig. 1b). In the young plantations (2 and 4 years), a mixture of Kudzu (*Pueraria phaseoloides*) and Desmodium (*Desmodium heterocarpon* subsp. *ovalifolium*) cover crops (C3 vegetation) have been implemented after planting. Cover crops were never used in the mature plantation (9 years), and in this case the soil was always kept bare after planting with oil palms. Accordingly, C3-derived SOC in the 9-year old plantation are attributed solely to oil palm-derived SOC.

Oil palm management leads to four well-defined management zones in productive plantations (about 4 years after establishment). In the study region, the weeded circle (WC) around the oil palm trunk is always kept free of vegetation (Fig. 1). At young ages (2 and 4 years old), all fertilizers are applied in WC. Associated with the beginning of harvest after 4 years, pruning starts and fronds are piled up in between palm trees (frond pile, FP). Each second avenue between palm lines becomes a harvest path (HP), where machines circulate. In productive plantations (after 4 years), fertilizers are evenly spread from the harvest path by machines, i.e. all management zone receive the same amount of fertilizers except the harvest path that receive none. The remaining area, especially the alternating avenue, represents the fourth zone, where the soil is kept bare and fertilization starts at 4 years old (interzone, IZ). The relative surface area of the four management zones were 60% (IZ), 18% (HP), 12% (WC) and 10% (FP). Fertilization depends on oil palm stand age. The 9-year old plantation received per hectare during the first year about 240 kg of NPK, 60 kg of Kieserite ($MgSO_4$), and 25 kg of KCl, of zinc and of boron. The amount of NPK increased over time while the use of other types of fertilizer varied from year to year. The year before sampling, 600 kg of NPK, 200 kg of phosphate rocks and 160 kg of KCl-MgO per hectare were spread in the plantation.

4.3.3 Soil and roots sampling

Plots of 1 ha were established in all plantations and savannas in areas with homogeneous soil, far from the influence of roads, rivers or groundwater, free of laterite formations (arecife) and without former amendment of compost or residues from processing oil mills. Five trees as replicates for management zones were selected randomly in each plantation. For each of the five replicate of trees, the adjacent management zones (FP, HP, IZ, and WC) were sampled on a systematic grid (Fig. 1). Frond piles, harvest paths and interzones were sampled at the same distance from trees (4.5 m) to assess the effects of management starting 4 years after establishment. Two additional points in the interzone were sampled at 2 and 3 m away from the trunk to assess the horizontal expansion of oil palm roots (IZ2, IZ3, Fig. 1). In the 2- and 4-year old plantations, only the weeded circles and interzones were sampled because of the absence of frond piles and harvest path in young plantations. In the 2-year old plantation, the point IZ3 was not sampled, as root densities were already low in IZ2 (Fig. S2). In the savanna plots, five sampling points were selected at regular distances along a 100 m transects.

Roots and soils were sampled with a cylindrical corer of 5 cm diameter at three depth intervals (0-10, 10-20 and 20-30 cm). Roots were separated from soil by sieving at 2 mm and rinsed to remove attached mineral particles. Fine roots that passed through the sieve were manually picked. For the 2- and 4-year old plantations outside of weeded circles, cover crop roots were removed from oil palm roots. Oil palm roots were divided into coarse roots (> 2 mm, corresponding to primary and secondary roots of oil palms) and fine roots (< 2 mm, corresponding to tertiary and quaternary roots of oil palms). Dry root biomass was determined after drying at 60 °C for 48 h. Soil samples were air-dried and sieved at 2 mm directly after collection and further oven dried at 40 °C for 48 h prior to laboratory analyses.

4.3.4 Soil analysis

Total C and nitrogen (N) contents in soil, as well as $\delta^{13}C$ signature were determined at the University of Göttingen with an isotope ratio mass spectrometer (Delta Plus, Finnigan MAT, Bremen, Germany). Because of the absence of carbonates in acidic soils, total C represents organic C. Residual water content was assessed by drying soil samples at 105 °C for 24 h. Bulk density was measured by inserting horizontally two cylinders of 100 cm^3 per depth at 0-5, 5-10, 10-20 and 20-30 cm depth in a soil pit located at the centre of each sampling plot. Bulk density in each cylinder was determined after drying at 105 °C and averaged between four cylinders for 0-10 cm depth interval and 2 cylinders for 10-20 and 20-30 cm depth intervals. Carbon stocks were calculated multiplying C contents with bulk density and the layer thickness and the respective fractions of C4 (savanna-derived C) and C3 (oil palm-derived) SOC. Soil available phosphorous (P) was determined using Bray II extraction method (Bray and Kurtz 1945). Three grams of soil were extracted by shaking for 15 min with 20 ml of 0.03 N NH_4F and 0.025 N HCl. Filtered extracts were mixed with a colorimetric reagent ($(NH_4)_6Mo_7O_{24}$ – $SnCl_2$) and absorbance was measured with a UV/VIS spectrometer at 660 nm (Lambda 35, Perkin Elmer, Buckinghamshire, United Kingdom).

4.3.5 C3 and C4 derived carbon

Relative portion of oil palm- and savanna-derived C in SOC were calculated based on the differences in $\delta^{13}C$ signature of biomass between savanna grassland, dominated by C4 photosynthetic pathway, and oil palms, C3 photosynthetic pathway, using two sources linear isotopic mixing model (Balesdent and Mariotti 1987):

$$f_{OP\_d} = \frac{\delta^{13}C_{S\_d} - \delta^{13}C_{NS\_d}}{\delta^{13}C_{OP\_b} - \delta^{13}C_{NS\_b}}$$

where f_{OP_d} is the fraction of oil palm (C3)-derived SOC, $\delta^{13}C_{S\_d}$ is the isotopic signature measured in a soil sample at depth d, $\delta^{13}C_{NS\_d}$ is the average isotopic signature at the corresponding depth in natural savanna reference sites, $\delta^{13}C_{OP\_b}$ is the averaged signature of oil palm fine roots in the mature oil palm plantation (mean = -28.2‰, standard deviation (SD) = 0.18, n = 9) and $\delta^{13}C_{NS\_b}$ is the average isotopic signature of the aboveground and belowground savanna biomass determined on representative subsamples of aboveground biomass collected on 1 m^2 and fine roots collected with soil cores at each sampling point (mean = -13. 6 ‰, SD = 1.1, n = 19). This approach assumes that the ^{13}C fractionation occurring during the integration of biomass into SOC at each soil depth is the same for savanna and oil palm biomass (Pausch and Kuzyakov 2012). No ^{13}C fractionation was observed between savanna plant biomass and savanna SOC in 0-10 cm layer (-13.7 ‰, SD = 0.5, n = 10).

Carbon stabilization per cumulative standing root biomass

Fine root stocks served as a proxy for the C input from oil palm roots. To account for differences in root stocks between management zones and root development time depending on the distance to the palm tree, cumulative standing fine roots biomass in each management zones was estimated by fitting a linear model on root biomass measured in various plantation ages. The model was integrated starting from plantation establishment for WC, but only starting from 4 years for IZ, FP and HP, i.e. when oil palm rooting system reached 4.5 m away from the tree (Fig. S2). Assuming constant belowground C input per unit of fine roots (Pausch et al. 2013), oil palm-derived SOC stocks were normalized per unit of cumulated fine root biomass to assess the effect of management zones on net C3 stabilization efficiency.

4.3.6 Soil incubation

Microbial biomass was analysed after incubation of topsoils (0-10 cm) from the 9-year old plantation (zones FP, HP, IZ and WC). Twenty grams of dry soil were rewetted to 60% WHC and incubated at 25 °C for 31 days. Jars were ventilated and weighed every week and rewetted once to compensate for the evaporated water. Carbon and nitrogen (N) in microbial biomass were measured at the end of the incubation by the fumigation-extraction method (Vance et al., 1987). For C and N contents, 5 g of incubated soil were fumigated for 24 h with ethanol-free $CHCl_3$ in a desiccator. Soils were extracted by shaking 1 h in 25 ml solution of 0.5 M K_2SO_4 and then filtered. Non-fumigated samples were processed in parallel. Total extractable organic C and N were analysed with a TOC-N analyser (Shimadzu, Kyoto, Japan). Extractable C in the non-fumigated samples was assumed to represent dissolved organic carbon (DOC). Microbial biomass C and N were calculated as the difference between fumigated and non-fumigated samples, which were used also to calculate microbial C:N ratio. Microbial biomass C was corrected by dividing extractable C with a factor of 0.45 (Beck et al. 1997), N with a factor of 0.54 (Brookes et al. 1985).

4.3.7 Basal respiration

Basal respiration of the rewetted samples was measured with the MicroResp^{TM} kit (Campbell et al. 2003). Three analytical replicates of 0.5 g for each field replicate were taken from the incubation jars after rewetting and incubated in 96 deep-well plate in parallel to the incubation in jars. Soil was kept moist by a moist paper towel fixed on the plate. Respiration was measured 1, 2, 4, 8, 15, 18, 24 and 31 days after rewetting using a MicroResp^{TM} kit (Campbell et al. 2003). To remove residual CO_2 in the wells, the plate was aerated with a fan before incubating for 6 h with the indicator plate on top (at 27 °C). The indicator plate was read before and after incubation with a spectrophotometer (Microplate reader BioTek SynergyMX) at 570 nm. Absorption calibration was done by dissolving a known amount of $NaCO_3$ with 1 M HCl in excess in closed jars with eight microwells of the indicator plate for 6 h (Campbell et al. 2003).

Soil respiration was partitioned using a two-pool mixed-model to describe SOC mineralization kinetics (Bonde and Lindberg 1988). The first pool follows a first-order decomposition kinetics, while the second follows a zero-order kinetics, corresponding to the stabilized basal respiration:

$$Cmin = C_l\left(1 - e^{-tk_l}\right) + BR\ t$$

where t is the time, $Cmin$ is the cumulative CO_2 mineralized to time t, C_l is the labile C pool released from sample preparation and re-wetting, k_l is the decomposition constant of the labile pool and BR is the basal respiration. The basal respiration of each field replicate was determined by fitting the model on the three analytical replicates. The metabolic quotient is the ratio of basal respiration over microbial biomass C (Cmic).

4.3.8 Statistical analyses

All statistical analyses were performed using the open source software R version 3.2.1 (R Core Team, 2016). One sampling point in WC and one in IZ were removed from all analyses due to very low $\delta^{13}C$ signatures, probably resulting from the former presence of C3 bush at these exact sampling locations. We used a linear mixed-effects model approach (“lme4” package), followed by Tukey HSD tests for post hoc pairwise comparisons, to test for the effects of management zones (FP, HP, IZ and WC) on soil parameters (bulk, C3 and C4 stocks, fine root biomass, net C3 stabilization, basal respiration, microbial biomass C and C:N ratio, and metabolic quotient) at each soil depth (0-10, 10-20 and 20-30 cm) in the mature plantation (9-year old) with palm trees as random factor. Normal distribution of residuals and homogeneity of variance were tested by Shapiro and Levene tests, respectively, and data was log-transformed if necessary. Causal relationships between parameters (C stocks *vs.* age, C3 stocks *vs.* fine roots) were assessed by linear regressions. Average C stocks at plot scale down to 30 cm depth were calculated using the relative surface area of each management zone. Associations among parameters were calculated using Pearson correlation. *p*-values were determined using the function *cor.test*.

A path analysis was performed to disentangle the direct and indirect effects of fine roots, soil microorganisms and nutrient application on the accumulation of oil palm-derived SOC by using the “lavaan” package (Rosseel 2012). Based on the priori knowledge, we developed an initial conceptual model that was both consistent with our data and which made biological sense (Fig. S3). We first created five conceptual groups of measured variables, which represented i) nutrient application (measure of available P), ii) fine roots C inputs (measure of fine roots biomass), iii) SOM quality (measure of C:N ratio of SOM), iv) SOC accumulation (measure of oil palm-derived SOC) and v) soil microorganism effects. As a proxy for the microbial effect, we used the score of each sample on the first axis (PC1 = 68 %) of a principal component analysis (PCA) including all microbial related variables (soil basal respiration, metabolic quotient, Nmic, Cmic, microbial C:N ratio) (Fig. S4). The conceptual model hypothesized that fine roots biomass and microbial activity have a direct impact on oil-palm SOC accumulation. Both might be affected by nutrient application. Additionally, microbial activity would be directly affected by root density through the amount of rhizodeposition and indirectly by changing organic matter quality. Finally, P availability could have a direct effect on microorganisms and SOM quality by changing the resources' stoichiometry in the soil. Pedoclimatic factors affecting SOC stabilization (e.g. soil mineralogy) were not specified in the model because they are identical between zones and cannot be modified by management. The adequacy of the model was determined by non-significant differences between the predicted and observed covariance matrices ($\chi 2$ tests, $p > 0.05$), low root mean squared error of approximation index (RMSEA < 0.1), high Tucker-Lewis index (TLI > 0.90) and high comparative fit index (CFI > 0.90) (Grace 2006; Rosseel 2012).

4.4 Results

4.4.1 Soil organic carbon stocks and origin

Observations done on the 9-year old plantation showed lower soil C stocks compared to the native savanna grasslands (Fig. 2). On average, 1.0 ± 0.2 kg C m^{-2} was lost down to 30 cm depth, considering the relative area of each management zone. However, spatial SOC distribution in the mature oil palm plantation depended on management zones. Soil C losses during that period of time in the top 10 cm under frond piles and, to a lesser extent, under weeded circles were lower than C losses in harvest paths and interzones (Fig. 2). This trend was similar down to 30 cm depth but the differences were not significant below 10 cm depth. Differences in SOC stocks between management zones arose mainly from a higher accumulation of oil palm-derived SOC (C3-derived) under frond piles and weeded circles (Fig. 2). Oil palm-derived SOC after 9 years already accounted for between 27% (IZ) and 45% (FP and WC) of the total SOC stock in the top 10 cm, where differences between zones were highest.

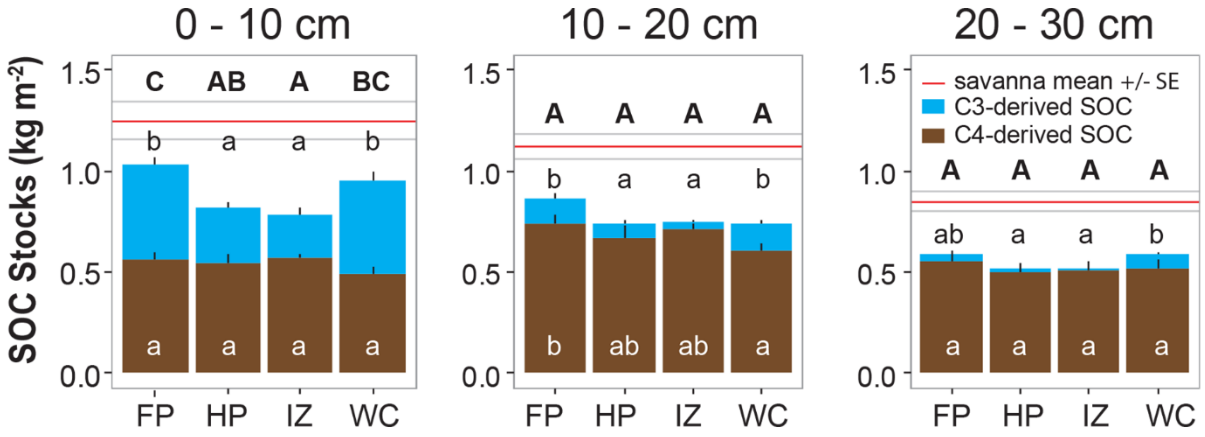


Figure 2. Soil organic C stocks separated between oil palm-derived SOC (C3-derived SOC) and savanna-derived SOC (C4-derived C) depending on management zones and soil depths after 9 years of oil palm cultivation on savanna grasslands. Mean values ± SE are represented (n=5 for FP and HP, n= 4 for IZ and WC). FP, front pile; HP, harvest path; IZ = interzone; WC, weeded circle. Letters indicate significant differences between management zones in total SOC (upper-case) and oil palm-derived SOC (black lower-case) and savanna-derived SOC (white lower-case). The red continuous line shows the original SOC level (± SE) in savanna grasslands (n=10). The difference between red line and the top of the stacked bars show the C losses over 9 years of oil palm plantations.

Below 20 cm depth, the contribution of oil palm-derived SOC dropped, accounting for 2% (IZ) and maximum 12% (WC) of the total SOC stocks. The amount of savanna-derived SOC (C4-derived) remaining after 9 years was lower in the top 10 cm than between 10-20 cm depth, indicating a faster decomposition of this C pool in the top soil compared to deeper soil layers. Nonetheless, management zones had little influence on the decomposition rates of savanna-derived SOC, except in 10-20 cm depth under weeded circles where more C was lost than under frond piles. A similar trend was observed in the top 10 cm that explains why total SOC under weeded circles was intermediate as compared to frond piles and harvest paths, despite the high amount of oil palm-derived SOC stabilized under weeded circles.

4.4.2 Root development and C inputs

Oil palm fine roots were first observed at 4.5 m away from palm trees in two out of the five investigated palms in the 4-year old plantations (Fig. S2). This indicates that oil palm rooting systems from adjacent palms started to overlap at that age, but root biomass at 4.5 m was still very low. Fine root biomass under weeded circles increased constantly during 9 years (Fig. 3a). At the age of 9 years, roots were observed in all management zones but root growth was strongly enhanced under frond piles, reaching the same fine root biomass as under weeded circles in only 5 years, well above fine root biomass in the inter zones and harvest paths (Fig. 3a).

Oil palm-derived SOC under weeded circles was highly correlated with fine root biomass (Fig. 3b). Oil palm-derived SOC stocks corresponded to 70 ± 5 % of fine root biomass stocks (slope = 0.70, $R^2 = 0.80$) at the time of measurement, independently of soil depth and plantation age. This percentage was relatively constant between the three soil depths; from 62 ± 10% in the top 10 cm to 50 ± 11% between 20-30 cm depth (Table 1). Oil palm-derived SOC accumulation per year was 3 to 4 times faster in the top soil 10 cm than in the underlying layers (Table 1).

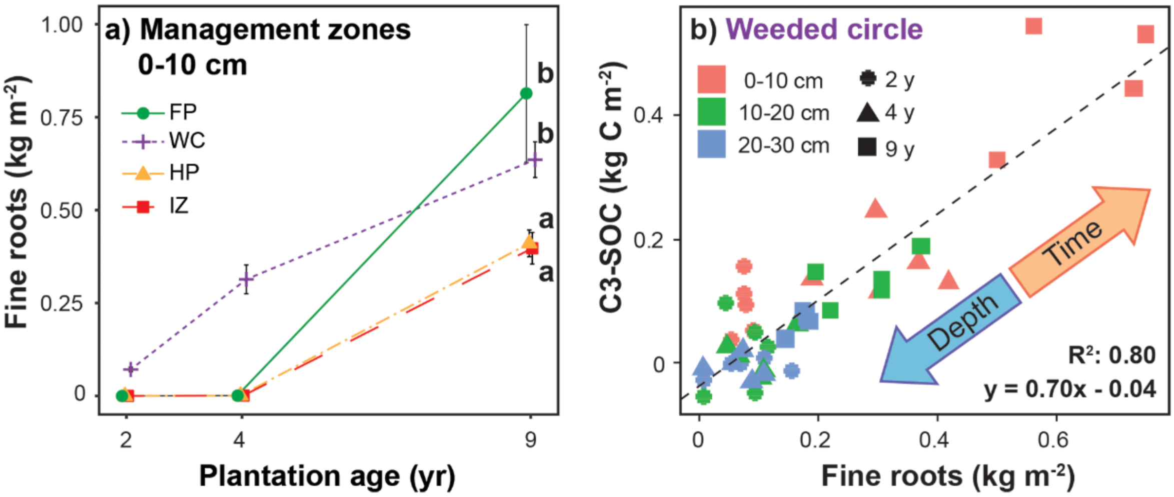


Figure 3. Oil palm fine root development and oil palm-derived SOC stocks. a) Oil palm fine root development with plantation age in the top 10 cm under frond piles (FP), weeded circles (WC), harvest paths (HP) and inter zones (IZ). Mean values ± SE are represented (n=5 for FP and HP, n= 4 for IZ and WC). Letters indicate significant differences between management zones in the 9-year old plantation; b) Relationship between oil palm-derived SOC (C3-SOC) and fine roots biomass under weeded circles. Overall linear regression is indicated by the dashed line. Negative C3-SOC values result from the natural variation standard of reference sites around the mean $\delta\ ^{13}$C value when C3-SOC accumulation is very low or absent. Negative data were not set to zero to avoid increasing artificially the mean of the respective depth and age, and thus decreasing the overall slope of the relationship. The two large arrows show the opposite effects of the plantation time and soil depth on the amount of new C (C3) stabilized in soil.

While oil palm-derived SOC in the weeded circle was accumulating from the beginning of the plantation, root biomass was measured at fixed time points. Oil palm fine root biomass was integrated over the whole duration of the plantation to calculate the cumulative fine roots biomass for each year and soil depth under weeded circles. Accordingly, oil palm-derived SOC accumulation corresponded to 14 ± 2%, 11± 2% and 11 ± 1% of the cumulated fine roots biomass stocks under weeded circles at 0-10, 10-20 and 20-30 cm depth, respectively (Table 1). Since soil depth had little impact on the relationship between oil palm-derived SOC and fine roots biomass, differences in oil palm-derived SOC stabilization rates between depths were mainly related to differences in fine roots biomass. The linear relationship across age showed that the proportion of oil-palm derived SOC stabilizing per amount of fine roots present in the plantation remains constant at least during 9 years (Fig. 3b).

Table 1. Oil palm-derived SOC stabilization under weeded circles at each depth depending on time, fine root biomass in the plantation and fine root biomass cumulated since plantation establishment (mean ± SE). All linear regressions were significant at $p < 0.001$

Weeded circles Depths *cm*	OP-derived SOC per year *g SOC m^{-2} yr^{-1}*	*R^2*	OP-derived SOC per fine roots *g SOC g^{-1} roots*	*R^2*	OP-derived SOC per cumulative fine roots *g SOC g^{-1} roots*	*R^2*
0-10 (n=14)	54 ± 6	0.85	0.62 ± 0.10	0.76	0.14 ± 0.02	0.86
10-20 (n=15)	18 ± 6	0.55	0.53 ± 0.11	0.60	0.11 ± 0.02	0.59
20-30 (n=15)	12 ± 2	0.75	0.50 ± 0.11	0.57	0.11 ± 0.01	0.80

Oil palm derived-SOC and fine root biomass were similar under frond piles and weeded circles in the top 10 cm in the 9-year old plantation (Fig. 3a). However, fine roots appeared at 4.5 m away from palms 4 years later than under weeded circles. To remove the effects of root density and duration of C inputs between zones, oil palm-derived SOC stocks were also divided by the cumulative roots biomass stocks and compared between management zones (Fig. 4).

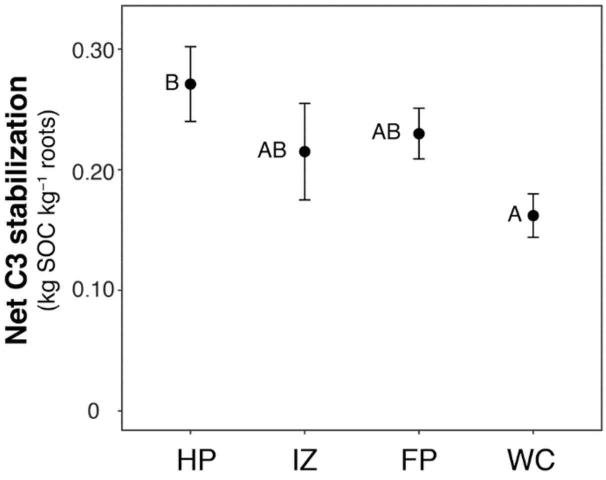


Figure 4. Net oil-palm derived SOC (C3) stabilized per amount of cumulated fine root biomass in each management zone after 9 years. Different letters indicate significant differences between harvest paths (HP), interzones (IZ), frond piles (FP) and weeded circles (WC). Mean values ± SE are represented (n=5 for FP and HP, n= 4 for IZ and WC).

After this normalization to the amount of fine roots biomass, the stabilization of oil palm-derived SOC was similar between frond piles and inter zones, two zones receiving the same amount of mineral fertilizers and sampled at the same distance to the tree. Consequently, only little C from the large amount of C present in dead fronds is eventually stabilized in SOC. The stabilization of oil palm-derived SOC was lower under weeded circles, the zone receiving the largest amount of mineral fertilizer, than under harvest paths, the only zone experiencing no direct application of mineral fertilizers.

4.4.3 Soil microorganisms and fertility

Microbial activity (basal respiration and metabolic quotient) and biomass (microbial biomass C and microbial C:N ratio) parameters were similar under frond piles and weeded circles (Fig. 5). In these two zones, the same amount of microbial biomass respired more C compared to harvest paths and interzones. Consequently, the metabolic quotient was highest under frond piles and weeded circles. Available P (Bray II) was an order of magnitude higher in weeded circles compared to the other zones, as expected by the higher fertilization application in that zone. Weeded circles also had the lowest amount of K_2SO_4-extractable C (DOC) despite high SOC content. (Table S1). Between the three zones (HP, IZ and FP) located at the same distance to the palm trees but varying in their management, P availability was two times lower in harvest paths (11.1 ± 0.3 µg g^{-1}) as compared to frond piles, but well above the P availability in native savanna sites (2.2 ± 0.3 µg g^{-1}), despite the absence of direct fertilization. The DOC amount was similar between the three zones and the C:N ratio was 1 unit higher under frond piles, indicating that only small change in SOC quality occurred between management zones.

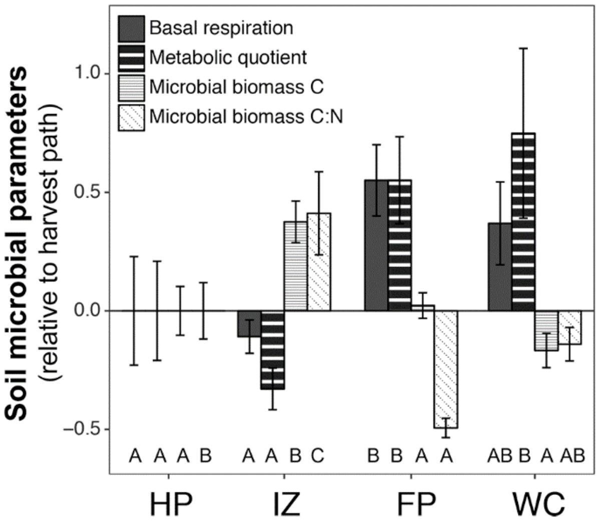


Figure 5. Relative effects of management zones on soil microorganisms in the 9-year old plantation: basal respiration, metabolic quotient, microbial biomass, and C:N ratio of microbial biomass. Microbial variables in interzones (IZ), frond piles (FP) and weeded circles (WC) were normalized with their respective mean value in the harvest path (HP), i.e. the zone receiving neither C inputs nor nutrient applications. Specific respiration (basal respiration divided by C content) is not represented because the effect of management zone was not significant. Error bars represent SE (n=5 for FP and HP, n= 4 for IZ and WC). Letters indicate significant differences between management zones for each parameter.

4.4.4 Drivers of the accumulation of oil palm-derived SOC

In the three zones located at the same distance to the palm trees (HP, IZ and FP), oil palm-derived SOC stocks (C3) were highly correlated with fine root biomass (r = 0.82), as well as to most microbial parameters, the amount of soil organic matter (C and N contents) and available P (Fig. 6). The basal respiration, the metabolic quotient and the microbial biomass N increased with higher oil palm-derived SOC but not with total C content, underlying the role of fresh organic C to maintain microbial activity. By contrast, higher C:N ratio in microbial biomass and, to a lesser extent, higher microbial biomass were associated to less oil palm-derived SOC and less fine roots biomass but with more savanna-derived SOC remaining after conversion to oil palm.

A path analysis was performed to disentangle the direct and indirect effects of fine roots, soil microorganisms and nutrient availability on the accumulation of oil palm-derived SOC at 4.5 m away from palm trees in the 9-year old plantation. Because of the high association among all microbial parameters (Fig. 6), the scores of each sample on the first axis (PC1 = 68 %) of a principal component analysis (PCA) including all microbial related parameters were used as a proxy for soil microorganisms in the path analysis (Fig. S4). The fitting parameters of the model were good ($P\chi^2 = 0.33$, RMSEA = 0.09, TLI = 0.97, CFI = 0.99), and the model explained 71% of the variance in oil palm-derived SOC (Fig. 7). Fine root biomass was strongly influenced by nutrient availability (available P) and was an important driver of microbial properties (i.e. increase in microbial biomass N, basal respiration, specific respiration and metabolic quotient and, in the opposite, decrease of microbial biomass C and C:N ratio see also Fig. 6). By contrast, nutrient availability had only a marginal ($p = 0.09$) and opposite effect on soil microorganisms. While fine root biomass influenced SOM quality (C:N ratio), microorganisms were not affected by SOM quality. The direct effect of fine roots on oil palm-derived SOC stabilization was 2.8 times stronger than the marginal effect ($p = 0.06$) of soil microorganisms. Accordingly, nutrient availability had an important indirect impact on the accumulation of oil palm-derived SOC by favouring root development and thereby C inputs without enhancing SOC mineralization.

4.5 Discussion

4.5.1 Drivers of SOC stabilization

Soil organic C stocks strongly varied depending on the management zones of the plantation. Specific management impacted the amount of new oil palm C input and its stabilization into SOC but had little effect on the decomposition rate of old savanna-derived SOC (Fig. 2). Carbon stabilization was mainly driven by C inputs from fine roots rather than by changes in C outputs from microbial mineralization (Fig. 7). The relationship between fine roots and oil palm derived SOC remained weakly affected by soil depth, plantation age and management zones (Table 1, Fig. 3 and 4). Fine roots biomass was a good proxy for soil belowground C inputs. Oil palm fine roots absorb nutrient and water, and therefore have definite growth and short-term self-pruning. In contrast, coarse roots, which have the function of conduction, have indefinite growth and long term self-pruning (Jourdan, Michaux-Ferrière, and Perbal 2000; Christophe Jourdan and Rey 1997). The relationship between cumulated fine root biomass and oil-palm derived SOC stocks would not remain constant once stocks reach equilibrium.

The relationship, however, was linear (Fig. 3), indicating that SOC stocks were still far from equilibrium after 9 years of cultivation and that oil palm-derived SOC will continue to increase.

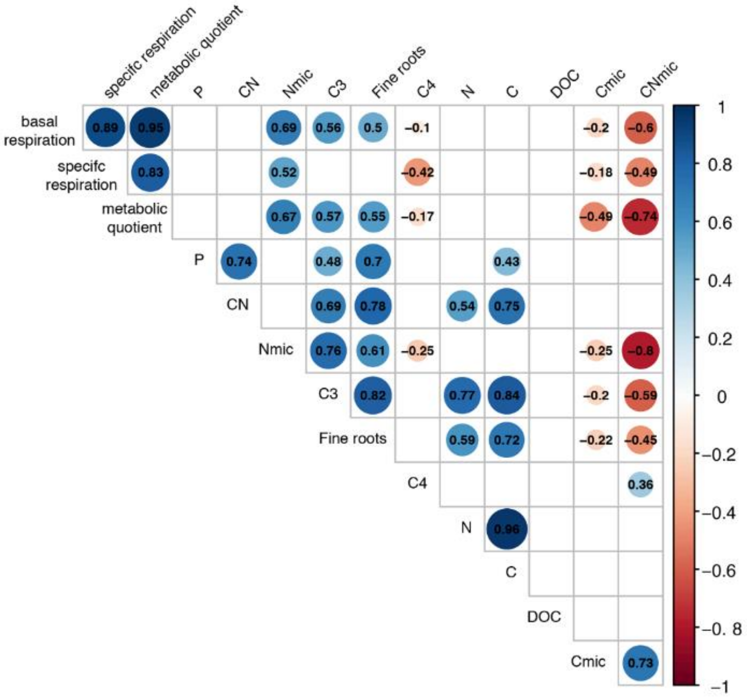


Figure 6. Pearson correlation matrix among soil variables in the top 10 cm. Correlation performed on samples collected in the three management zones located at 4.5 m away from palm trees (HP, IZ, and FP). Variables are: basal respiration, specific respiration, microbial quotient, oil palm-derived (C3) and savanna-derived (C4) SOC stocks, soil C and N contents (C, N), microbial biomass C and N (Cmic and Nmic), C:N ratios of SOM (CN) and microbial biomass (Cmic, Nmic), K_2SO_4-extractable C (DOC), and fine root biomass stocks (Fine roots). Only significant correlations are represented ($p < 0.05$, n= 14).

Microbial metabolism and biomass were strongly affected by fine roots. Higher organic C availability in terms of oil palm-derived SOC and C inputs under frond piles and in the weeded circle were associated with microbial communities characterized by a high mineralization activity but of low efficiency – the so termed *r* strategy (Loeppmann et al. 2016). The high metabolic quotient results from either a low C use efficiency or a high microbial biomass turnover. By contrast, zones with low C availability such as the harvest path and the interzones were associated with microbial communities more efficient to maintain their biomass despite lower mineralization rates. Their lower metabolic quotients and their higher C:N ratios suggest that the scarcity of C increased the proportion of K strategists and fungi within microbial communities (Mouginot et al. 2014; Six et al. 2006). While the main effect of nutrient availability on microbial communities was indirect by increasing roots C inputs, it tended to have also a minor direct but contrasting effect on microbial communities. Lower C to nutrients ratio favours high C use efficiency of microorganisms, which would explain the larger microbial biomass in interzones despite the low microbial activity and roots density (Sinsabaugh et al. 2013).

Nonetheless, management impacts on microbial communities and their resource consumption strategies levelled-off resulting in similar fraction of SOC mineralized in all management zones. Weeded circles were the only area of the plantation showing slightly lower net SOC stabilization and savanna-derived SOC stocks (Fig. 2 and 4). The very high amount of fertilizer applied already in the early stage of the plantation might have slightly fastened SOC turnover. Nonetheless, management effects were small in regards to the 10-fold increase of P availability in this zone as compared to the rest of the plantation. The fact that nutrient availability had little impact on the SOC mineralization and stabilization indicates that microorganisms were mostly C-limited. Indeed, soils were depleted in SOC and the whole surface area of the plantations, even areas not directly fertilized, experienced an increase in P availability as compared to native savannas.

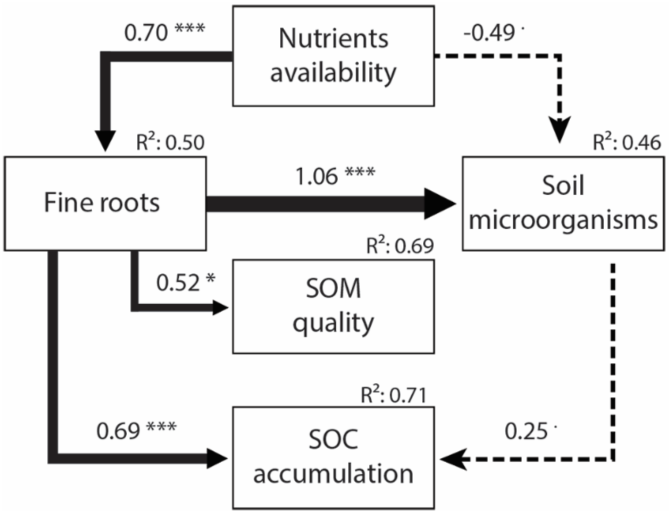


Figure 7. Drivers of oil palm-derived SOC accumulation in the top 10 cm of the three management zones located at 4.5 m away from palm trees (HP, IZ, and FP). Phosphorus availability was used as proxy for nutrient availability. Fine roots correspond to fine root biomass stocks. Scores of samples on the 1st axis (68 %) of a PCA of all microbially related variables were used as proxy for microbial effects. The C:N ratio of SOM was used as proxy for soil organic matter quality. Solid arrows represent significant effects (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n= 14) and widths are proportional to the effect. Dashed arrows represent marginally effects ($p < 0.10$). Non-significant relationships ($p > 0.10$) are not represented (Nutrient availability to SOM quality and SOM quality to soil microorganisms).

In summary, management and its impact on soil belowground C inputs and nutrient availability did not lead to priming of recalcitrant SOC nor to faster turnover of fresh organic SOC, except around palms in the weeded circles. Hence, SOC distribution was not driven by an altered decomposition of SOC pools but by different rates of fine root growth and the resulting soil C inputs depending on management.

4.5.2 Drivers of soil C inputs

Oil palm rooting system follows a relatively rigid and genetically determined development (Jourdan et al. 2000; Christophe Jourdan and Rey 1997). Primary lateral roots start to grow one year after germination at a rate of 3 mm d^{-1}, confirming that roots of palms, which are planted a year after germination, reach 4.5 m in the fourth year of a plantation (Christophe Jourdan and Rey 1997). Nevertheless, roots development showed plasticity, reacting to management as shown by the fast development of fine roots under frond piles. Root development is generally enhanced in nutrients-rich zones (Hodge 2004). Phosphorous availability was an important driver of root development, favoring soil C inputs from fine roots, and consequently the accumulation of new SOC (Fig. 7). Its distribution did not exactly reflect the pattern of fertilizer application (Table S1). Despite no direct fertilization applications, harvest paths were enriched in available P as compared to the reference savanna sites and only frond piles exhibited a significantly higher P availability. This discrepancy may result from the initial soil preparation with dolomite and phosphate rocks and seems to be maintained over time by the recycling of organic P inputs from rhizodeposition, root turnover, and frond mineralization.

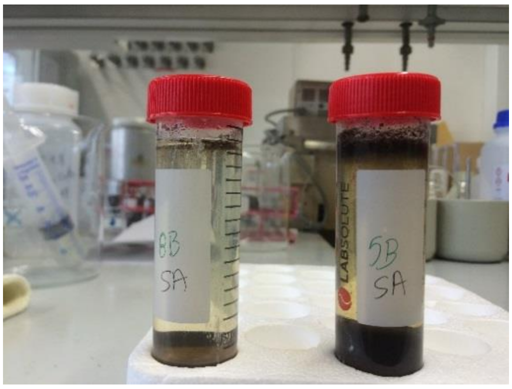
The positive impact of frond piles on root development is likely not limited to P availability. Fronds piles increase the availability of major cations (Law et al. 2009). Similarly, it was shown that the application of empty fruit bunches on the surface increases soil moisture (H.-H. Tao et al. 2016) and roots development (Kheong et al. 2010). The positive impact of frond piles on SOC stocks has been previously reported (Haron et al. 1998; Law et al. 2009) but authors have already highlighted the small increase of SOC stocks given the huge amount of frond’s biomass C concentrated on a small surface area (2-3 kg C m^{-2} yr^{-1} in frond piles). It was suggested that fronds were mineralized mostly aboveground with little contribution to SOC (Haron et al. 1998). Our findings confirm this hypothesis and indicate that the positive impacts of frond piles arise more from the improvement of soil conditions (likely nutrient availability, humidity, protection from erosion) that favours root growth than from their role as a C source.

4.5.3 Increasing SOC stocks

Soil organic C stabilization depends on belowground C inputs and not on the management induced variation of SOC mineralization rates (Fig. 7). This field evidence-based finding is similar to the conclusion of a modelling study on arable cropping system in temperate zones (Autret et al. 2016). Soil C inputs should be enhanced to increase soil C sequestration in oil palm plantations. The current management practice that consist in piling dead fronds, however, is not efficient to integrate the fronds’ organic matter into the soil. This can be explained by several mechanisms. First, organic matter quality in fronds is low due to high C to nutrient ratios (Yusuyin et al. 2015). This decreases the C use efficiency of microorganisms. Second, bioturbation from soil fauna is limited as indicated by a sharp transition between decomposing fronds and the soil surface observed in the field. Soil fauna abundance, especially of earthworms, and biogenic macroaggregates are lower in oil palm plantations as compared to native savanna and in improved pastures (Lavelle et al. 2014a). Consequently, fronds’ organic matter does not benefit from the protection mechanisms that minerals would provide if it was integrated into the soils by faunal activity (Schmidt et al. 2011). Application of composted mill residues would be a solution to decrease C to nutrient ratios of litter and improve the integration of organic matter into the soil by favouring soil fauna activity, humification and mineral protection.

The availability of organic residues from the palm oil production chain that could be applied in plantations, however, is limited. Leguminous cover crops, as already implemented in the younger plantations, are an alternative to increase aboveground and belowground C and N inputs. Enhancing root growth by mimicking the effects of frond piles is a promising solution to increase SOC if palms have plasticity to allocate more C to their rooting system when soil conditions are favourable or if palm varieties are developed for that purpose. Oil palm fronds could be spread on larger surface area or mixed with other residues, such as empty fruit bunched or fibers, to make a mulch. Even solutions not based on organic matter that would limit soil evaporation, retain nutrients, limit run-offs and erosion might improve root development. Future research should address whether SOC stocks eventually recover to initial SOC levels. Ensuring long-term soil fertility in oil palm plantations is fundamental to avoid a conversion of natural ecosystem constrained by soil degradation in older plantation. The benefits from increasing SOC in terms of climate change mitigation would cascade far beyond the amount of C sequestrated in the soil because of the gain in soil fertility.

5 Manuscript 4: Long-term changes in soil organic carbon fractions and natural ^{13}C abundance following pastures conversion into oil palm plantations



*“**Life** is like **riding** a **bicycle**. To **keep** your **balance** you must keep **moving**”*

Juan Carlos Quezada, Christopher Poeplau, Alexandre Buttler, Thomas Guillaume

Status: Under preparation

5.1 Abstract

Planted pastures conversion is a deforestation-free land use change option that merit attention to reduce the drastic impacts of agriculture expansion in tropical forested areas. In recent decades, pastures established on former savanna areas have been one of the primary sources of land for the expansion of important perennial crops, yet today, the soil biogeochemical impacts of this conversion remain poorly quantified. OP plantations comprise a large portion of the new land cover type in savannas and pastures areas, particularly in Colombia. This conversion of C4 (pastures) into C3 vegetation (OP plantations) allows to investigate and understand better the dynamics of SOC in terms of source and turnover-times. Also, deeper understanding of SOC stabilization and its dynamics can be achieved if its various pools or fractions are investigated. Here, we combined a physico-chemical fractionation method, which separated SOC into five fractions: dissolve organic C (DOC), particulate organic matter (POM), SOC attached to silt and clay (S+C), SOC attached to sand and stable aggregates (S+A) and chemically resistant (rSOC), and natural ^{13}C abundance in a unique long-term 56-years old pasture derived chronosequence of OP plantations to elucidate potential mechanisms underlying SOC dynamics in the pastures to OP conversion LUC. Contrary to our expectations, we did not find an overall shift in the size and distribution of SOC pools. A rather, V-shape response characterized the dynamics of C concentration, SOC distribution and mass contribution over the entire period of OP cultivation, including the effect of LUC, in the POM, rSOC and S+C fractions. This means that these SOC fractions responded negatively to pasture conversion into OP in the short-term, first cultivation cycle, but thereafter this negative trend was reversed. The POM fraction was the most affected fraction by LUC during the first OP cycle where almost half of the initial SOC was lost. The recovery of C concentration in POM was due to the linear increase of OP-derived POM and no change of pasture-derived POM over time. When only OP cultivation time was considered and not pasture conversion, the S+A displayed the fastest decay rate among all fractions with a very short half-life time of 5.8 year. This was due to the fast decline of both pasture- and OP-derived C. Pastures conversion into OP have different short- and long-term implication, not only in terms of C sequestration, but also for soil fertility. Soil C sequestration would be benefited by the recovery of C content in more stable fractions like rSOC and S+C, whereas short-term fertility would be positively affected by the recovery of the POM labile fraction.

5.2 Introduction

The role of agricultural soils in climate change mitigation and food security enhancement was recently acknowledged by policymakers, who launched the “4 per 1000” initiative during the COP21 (Lal 2016). The initiative’s rational is that an increase of 0.4% of soil organic carbon (SOC) content per year down to 40 cm depth would compensate for anthropogenic greenhouse gas emissions and concomitantly improve various soil properties. Nonetheless, increasing C sequestration in agricultural soils necessitates deep understanding of the mechanisms involved in SOC stabilization upon management practices and land use change (LUC). Among the numerous pathways of agricultural expansion in the tropics, forest conversion into oil palm (OP) plantations has received special attention due to the vast spread of this type of agroecosystem in high conservation value areas and because deforestation often leads to changes in SOC storage. Despite the extensive research conducted on forest conversion to OP, large uncertainties remain about the mechanisms controlling SOC stock changes.

Tropical forest conversion into OP does not imply a change in the vegetation’s photosynthetic pathways, thereby forest soils do not exhibit the related relatively large change in their isotopic composition with time. Conversely, a replacement between C_3 and C_4 vegetation or vice-versa, causes a relatively large shift in the isotopic signature of soils that can be used as a tracer to better asses SOM dynamics. Indeed, the use of natural $\delta^{13}C$ abundance have proven efficacy to quantify and trace the fate of old and new SOC as a consequence of LUC. More specifically, in the case of pastures conversion into OP, a shift of C_4 into C_3 vegetation takes place and therefore it is possible to obtain novel insights into the dynamics of SOC as a consequence of OP cultivation. To our knowledge, only a handful of studies, so far, have reported on the effects of converting non-forested areas into OP on SOC stocks and other soil biogeochemical properties (Frazão et al. 2013; Goodrick, P. N. Nelson, et al. 2015; Quezada et al. 2019; Rüegg et al. 2019; Wakelin et al. 2016). Of those studies, even fewer have taken advantage of the natural abundance $\delta^{13}C$ approach. In those studies, the net balance between old C_4-C decomposition and new OP-derived C accumulation have resulted in increases, decreases and no change in total SOC stocks. Such variable SOC changes, in all cases, is explained by the magnitude and direction of change of old C_4-derived C. While in two studies grassland- and savanna-derived C were highly resistant to decomposition (Goodrick et al. 2015; Quezada et al. 2020 unpublished), in two others pasture- and savanna-derived C stocks declined very sharply during the first rotation cycle of OP cultivation (up to 30 years) (Quezada et al. 2019; Rüegg et al. 2019). Such contradictory and scarce data call for further research on SOC cycling when C4 soils are converted into OP.

Deeper understanding of SOC stabilization and its dynamics can be achieved if its various pools or fractions are investigated. It is widely accepted that SOC is highly heterogeneous and composed of many fractions differing in their cycling rates and degrees of stabilization (Six et al., 2002; von Lützow et al., 2006; Grandy and Robertson, 2007). Fractions are operationally-defined by methods based on chemical, size, aggregation and density properties (von Lützow et al. 2007). Partitioning of SOC into pools can be performed by using different chemical, physical or combined fractionation schemes. To date, numerous fractionation schemes have been applied to elucidate mechanisms controlling SOC storage and C cycling following LUC. Also, it is widely accepted that an effective long-term soil C sequestration mechanism, should preferably store OM in more stable C pools with longer residence time, i.e. mineral associated fractions (Chenu et al. 2019).

Surprisingly, within the large body of research about OP plantations expansion in the tropics, no study so far has applied any kind of SOC fractionation scheme. Furthermore, in the existent literature no consistent trends are found in the response of SOC to LUC into OP. While the majority of publications have reported reduced SOC stocks (Chiti et al. 2014; Dislich et al. 2017; Guillaume et al. 2015; van Straaten et al. 2015) few others have reported increases or no change in SOC stocks (Frazão et al. 2014; Rahman et al. 2018b; Smith et al. 2012). This variation in the response of total SOC stocks, could be better explained by examining the redistribution of total SOC into its different fractions following LUC. For example, by using the same soil samples that we included in this work, Quezada et al. (2019), reported that SOC stocks declined initially up until 36 years of OP cultivation and then stabilized for the remaining time period of the second OP cycle. These authors noted that such combined trend in total SOC stocks in the full soil profile (0-50 cm) was explained by the different pattern's dynamics that OP- and pasture-derived SOC exhibited in the five sampled soil layers down to 50 cm. While in the topsoil layer (0-10 cm) a stabilization phase followed an initial declined of total SOC due to a remarkable loss of original pasture-derived C that was partly compensated by new OP-derived accumulation, in the subsoil layer (30-50 cm) SOC stocks declined linearly without any sign of stabilization. It is clear that the conversion of pastures into OP plantations can be considered as a long-term plant labeling experiment and thus an ideal model to assess stabilization, decomposition and turnover of SOC in fractions with fast, medium and slow turnover under field conditions.

In this paper, we combined a physico-chemical fractionation method and natural ^{13}C abundance in a unique long-term 56-years old pasture derived chronosequence of OP plantations located in the savanna region of Los Llanos in Colombia. By using these two methodological approaches, simultaneously, we aim to bridge some knowledge gaps in the assessment of OP expansion on SOC dynamics. More specifically we seek to: 1) determine which SOC fractions are the most affected by pasture conversion into OP, 2) determine the ^{13}C isotopic composition of C pools and 3) quantify fundamental parameters of SOC fractions dynamics like stabilization and decomposition rates, time necessary to reach steady state of stocks and mean residence time (MRT). We hypothesized that pastures conversion into OP will deplete the δ^{13}C isotopic signature of all measured fractions, but the isotopic changes between fractions will follow different dynamics, i.e. linear or first-order decay. The most labile fractions like particulate organic matter (POM) will be the most responsive to pasture conversion and therefore will have the fastest turnover rates (0-15 years following LUC). Also, we expect to observe an overall shift in the size and distribution of SOC pools, i.e. pastures will have a greater proportion of total

SOC in the form of more labile fractions, while OP plantations will exhibit a greater proportion of C associated with more resistant fractions like the mineral silt + clay fraction.

5.3 Material and methods

5.3.1 Study sites

The study area was located at Los Llanos region of Colombia, in the large-scale farm of La Cabana located close to the town of Cumaral in the Department of Meta, Colombia (73°22'W, 4°16'N) with mean annual temperature and precipitation of 27°C and 3400 mm, respectively. Detailed information about the study area including weather and a general description is presented in Quezada et al. (2019). Prior OP establishment, all plots were sown pastures of *Brachiaria spp.* Selected OP plots represented first (12-, 18-, 30-years old) and second rotation cultivation cycles (32-, 45- and 56-years old). Three pastures located in the surrounding area of the commercial farm were sampled as reference sites. Soils in this OP chronosequence were Inceptisols (Oxic Dystropepts) of about 70 cm depth overlaying alluvial sediments.

5.3.2 Samples collection and soil fractionation

Soil samples for fractionation were obtained between 2016 and 2017 by a transect method to capture spatial heterogeneity(Nelson et al. 2015), as describe in Quezada et al. (2019). To summarize, three 50-m diagonal transects were traced at each of the plots, except in the 12- and 32-year OP plots where there was only one transect. At each transect, soil samples were taken every 2.5 m with a 6-cm diameter auger from 0 to 50 cm depth, but for the purpose of this study only samples from the 0-10 and 30-50 cm depths were used. Samples collected from the same soil depth at each transect were combined and thoroughly mixed. After field collection soils were air-dried, manually crushed, sieved through a 2-mm sieve, air dried and stored in sealed plastic bags until transportation to Switzerland. Upon arrival at the laboratory in Switzerland, soils were oven dried at 35°C and stored at room temperature. Prior to conducting the fractionation procedure, soil biological and physicochemical properties were determined (reported in Quezada et al., 2019).

Soil physicochemical fractionation was performed by following the approach proposed by (Poeplau and Don 2013; Zimmermann et al. 2007), where five SOC fractions related to the Rothamsted C model (RothC) are isolated (Fig. 1). The obtained fractions were as follows: two active, POM and dissolved organic carbon (DOC); two slowly cycling, SOC attached to silt and clay particles without being chemically resistant (S+C), SOC attached to sand and stable aggregates (S+A) and one passive fraction, chemically resistant (rSOC). First, 30 g of oven dried soil was dispersed ultrasonically using an energy input of 22 J ml^{-1} during 112" (Sonifier 450-D, Branson, Danbury, USA). Then, the dispersed suspension was separated into coarse, fine and DOC fractions by wet sieving using a 63 µm sieve with 2300 ml of deionized water. In a centrifuge tube, 35 ml of sodium polytungstate (SPT) solution adjusted to 1.8 g cm^{-3} was added to the > 63 µm fraction to separate the POM and the S+A fractions. The mixture was gently mixed with a spoon and centrifuged at 4700 RPM by 15′. Following centrifugation, the S+A fraction remained at the bottom of the centrifuge tube and the floating POM (< 1.8 g cm^{-3}) was collected in 50 µm seed bags. S+A and POM fractions were rinsed with deionized water to remove the excess SPT. The < 63 µm suspension, was vacuum-filtered using a milipore filter (0.45 µm) and an aliquot of the filtrate was taken and frozen to determine DOC. To obtained the S+C fraction, the < 63 µm mixture was centrifuged at 4700 RPM by 20′, dried overnight at 40°C and weighted. Finally, the rSOC fraction resulted after oxidizing for 18 hours at 25°C with 6 % NaOCl, a 1 g subsample of the S+C fraction. The oxidation product was centrifuged at 4700 RPM by 15′, decanted, washed and centrifuged again. All the obtained solid fractions (4) were dried at 40°C and weighted.

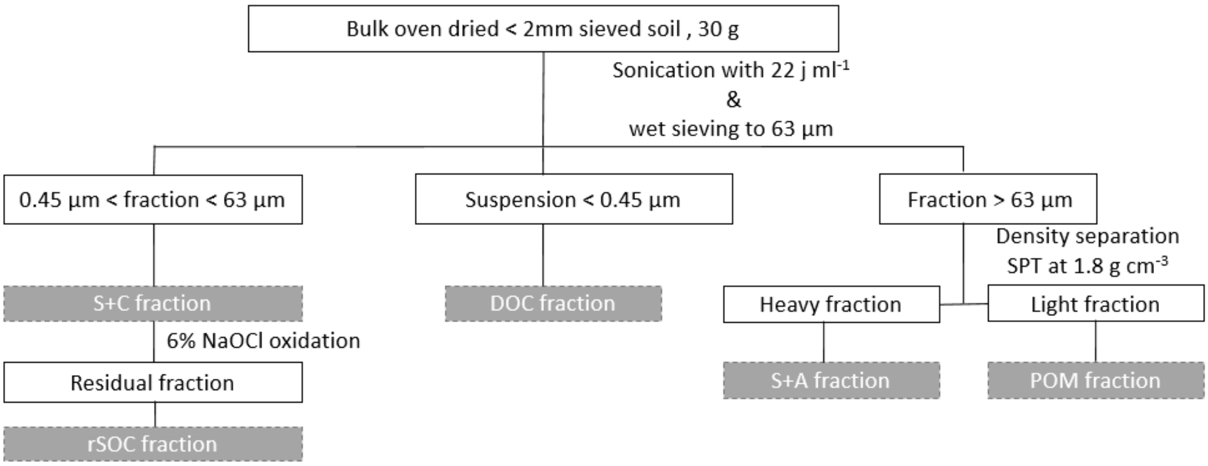


Figure 1. Schematic representation of the used SOC fractionation scheme (after Zimmermann et al., 2007). S+C = silt and clay, rSOC = resistant, DOC = dissolve organic C, S+A = sand and stable aggregates and POM = particulate organic matter.

C content and isotope analysis

For total C and relative isotope C abundance in the POM, S+C, rSOC and S+A fractions, samples were homogenized, ball milled, loaded in tin cups and analyzed with an elemental analyzer coupled to an Isotope Ratio Mass Spectrometer at the stable isotope lab at the Swiss Federal Institute for Forest, Snow and Landscape Research WSL, Birmensdorf, Switzerland. DOC was determined by thermal oxidation with a liquid analyzer. As soil pH was acidic, no carbonates were expected, so that the total C content corresponds to SOC. The $\delta^{13}C$ signature of samples were expressed in δ notation with units of per mil (‰), relative to the international Pee Dee Belemnite (PDB) limestone standard.

A mass balance equation (Balesdent and Mariotti, 1996) was used to determine the proportion of OP- and pasture-derived C (*f*) in each fraction:

$$f = (\delta^{13}C_{sam} - \delta^{13}C_{past})/(\delta^{13}C_{OP} - \delta^{13}C_{past}) \qquad (1)$$

where $\delta^{13}C_{sam}$ was the $\delta^{13}C$ signature of the sampled soil fraction, $\delta^{13}C_{past}$ was the $\delta^{13}C$ signature of the corresponding soil fraction in the reference pasture sites and $\delta^{13}C_{OP}$ was the $\delta^{13}C$ signature of OP biomass determined from OP fine root biomass $\delta^{13}C$ signature. OP-derived C concentration was calculated by multiplying the C content of each fraction by the proportion of OP-derived C obtained with equation 1 and pasture-derived C concentration was the difference between OP-derived C concentration and C concentration in each fraction.

5.3.3 Statistical analysis

All statistical analyses were carried out using R software version 3.4.0 (R Development Core Team, 2017). One-way analysis of variance (ANOVA) was used to examine the effects of pastures conversion into OP on SOC fractions' characteristics including C concentration, relative proportion of C in each fraction and soil fraction masses. To do this, OP plantations were grouped by OP cycle as follows: the 12-, 18- and 30-years old as first cycle plantations (*n*= 6) and the 32-, 45- and 56-years old as second cycle plantations (*n*= 7). Pastures were considered as the reference land use (*n*= 3). Following ANOVA statistical significance, the "multcomp" function was used to perform multiple comparisons of Least Significance Difference (LSD) between the three groups. Normal distribution was checked for all analysis according to the Shapiro-Wilk's test.

To assess the relationships between SOC fractions' characteristics and OP cultivation time linear and nonlinear regression analysis were performed using only OP sites. The non-linear model consisted in a single exponential decay model

$$y = c * \exp(-k * t) \tag{2}$$

where *y* is the stock of C, *c* is the initial pasture C stock before OP cultivation, *k* is the first-order decay rate constant and *t* is time after pasture conversion.

For this the nonlinear least square "nls" and linear model "lm" functions in R were used. Best model were based on the lowest Akaike information criterion (AIC) and the highest coefficient of determination (R^2) values to select the best model to represent the fractions' dynamics over time. Regression analysis did not consider the effect of LUC as in the case of ANOVA analysis, thus pastures data were not included. While for most variables a linear model was selected (Supplementary TableS1), for C concentration in the S+A fraction and pasture-derived C dynamics over time in the rSOC, S+C and S+A, the best fit model was a single exponential decay model (Eq. 2).

When applicable the half-life (HF) of the pasture-derived C in each fraction was calculated from Eq. 2 according to Eq. (3).

$$HL = \ln(2) / k \tag{3}$$

For all data, differences reported here are statistically significant at $P \leq 0.05$, unless stated otherwise.

5.4 Results

5.4.1 Distribution of SOC fractions mass

An average of 96.8 % of whole soil mass was recovered in soil fractions. The overall distribution of SOC fractions (POM, S+C and S+A) as a portion of the whole-soil mass was altered when OP plantations replaced former pastures in the topsoil layer (0-10 cm). Despite the small contribution of POM mass to total whole-soil mass (< 1% in general) at all sites, this fraction was the most affected by LUC. POM mass decreased ($P \leq 0.001$, Table 1) after pasture conversion at same levels in the first and second OP cultivation cycles. However, pasture conversion resulted in different changes in the S+C and S+A fractions when comparing to the first and second OP cultivation cycles. While the S+C fraction decreased in the first OP rotation cycle, it increased again in the second cycle, S+A increased initially and then decreased in the second OP rotation cycle (but the differences were only significant between first and second OP rotation cycles) ($P \leq 0.001$, Table 1).

Table 1. C concentrations, SOC distribution and mass contribution of soil fractions in pastures and two OP rotation cycles.

SOC fraction	C concentration (g kg^{-1})			SOC distribution (% whole soil SOC)			Mass (g)		
	Pastures	OP cycle 1	OP cycle 2	Pastures	OP cycle 1	OP cycle 2	Pastures	OP cycle 1	OP cycle 2
DOC	0.35 (0.04)a	0.29 (0.03)a	0.22 (0.02)b	1.44 (0.08)	1.75 (0.16)	1.46 (0.13)	NM	NM	NM
POM	2.07 (0.45)a	1.06 (0.15)b	1.63 (0.20)ab	8.35 (1.27)ab	6.57 (1.25)b	10.47 (0.56)a	0.29 (0.09)a	0.09 (0.01)b	0.15 (0.02)b
rSOC	5.59 (0.47)a	3.57 (0.35)b	4.09 (0.46)ab	23.45 (3.39)	21.57 (2.31)	24.47 (1.08)	NM	NM	NM
S+C	10.19 (0.65)a	6.43 (0.73)b	8.98 (1.05)ab	42.01 (2.74)ab	38.79 (4.53)b	54.06 (3.12)a	15.26 (1.56)ab	11.05 (0.78)b	20.31 (2.12)a
S+A	6.29 (1.90)	6.93 (2.24)	0.99 (0.32)	25.16 (6.47)ab	37.92 (10.77)a	6.30 (2.46)b	14.02 (1.25)ab	18.14 (0.79)a	8.89 (1.99)b

Mean values followed by same letter for a SOC fraction at a given measured fractions' property did not differ significantly after post-hoc LSD test ($p \leq 0.05$)

Standard errors are in brackets. NM: value not measurable

During the 56 years of OP cultivation after pasture conversion, there was a shift in the mass distribution of SOC fractions. Whole-soil mass proportion at OP sites was dominated either by S+C or S+A depending on stand age (Fig. 1). Major changes in mass proportions occurred between these two mineral fractions. The mass loss rate of 0.36 g C yr^{-1} in the S+A fraction was compensated by the mass gain of 0.37 g C yr^{-1} in the S+C fraction. This redistribution of S+C and S+A caused that the S+C fraction became the predominant fraction in the oldest OP plantations (86% of total mass, derived from Fig. 1), whereas the S+A fraction was the most abundant for the youngest first-cycle plantations (64%, derived from Fig. 1). In contrast to the observed decrease in POM mass due to LUC, the POM mass increased linearly at 0.002 g C yr^{-1} (Fig. 1) with OP cultivation time during the first and second OP cultivation cycle.

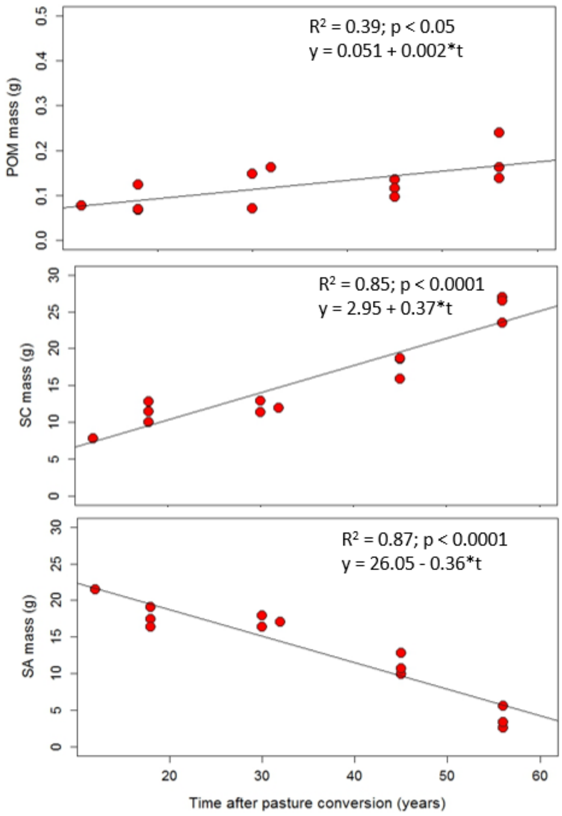


Figure. 1. SOC fractions mass dynamics during OP cultivation time in the topsoil layer (0-10 cm). Black lines are the fitter linear regressions.

5.4.2 Soil carbon dynamics in fractions

SOC concentrations in POM, rSOC and S+C exhibited similar patterns following LUC into OP. In these three fractions, SOC concentration decreased ($P \leq 0.05$, Table 1) after pasture conversion and then increased again at the second OP cycle (but differences to pastures and OP cycle 1 were not significant). SOC concentration in the DOC fraction did not differ between pastures and the first OP rotation cycle, but then declined at the second OP cycle ($P \leq 0.05$, Table 1). For the S+A fraction, SOC concentration remained stable following LUC to the first OP cultivation cycle and then decreased, although not significantly ($P \leq 0.07$, Table 1), in the second OP cycle. The dynamics of the portion of total SOC in all fractions generally mirrored the shift in the fractions' mass distribution and C concentration (Table 1), meaning that the major portion of total SOC was associated with the S+A and S+C in the youngest and oldest OP plantations, respectively.

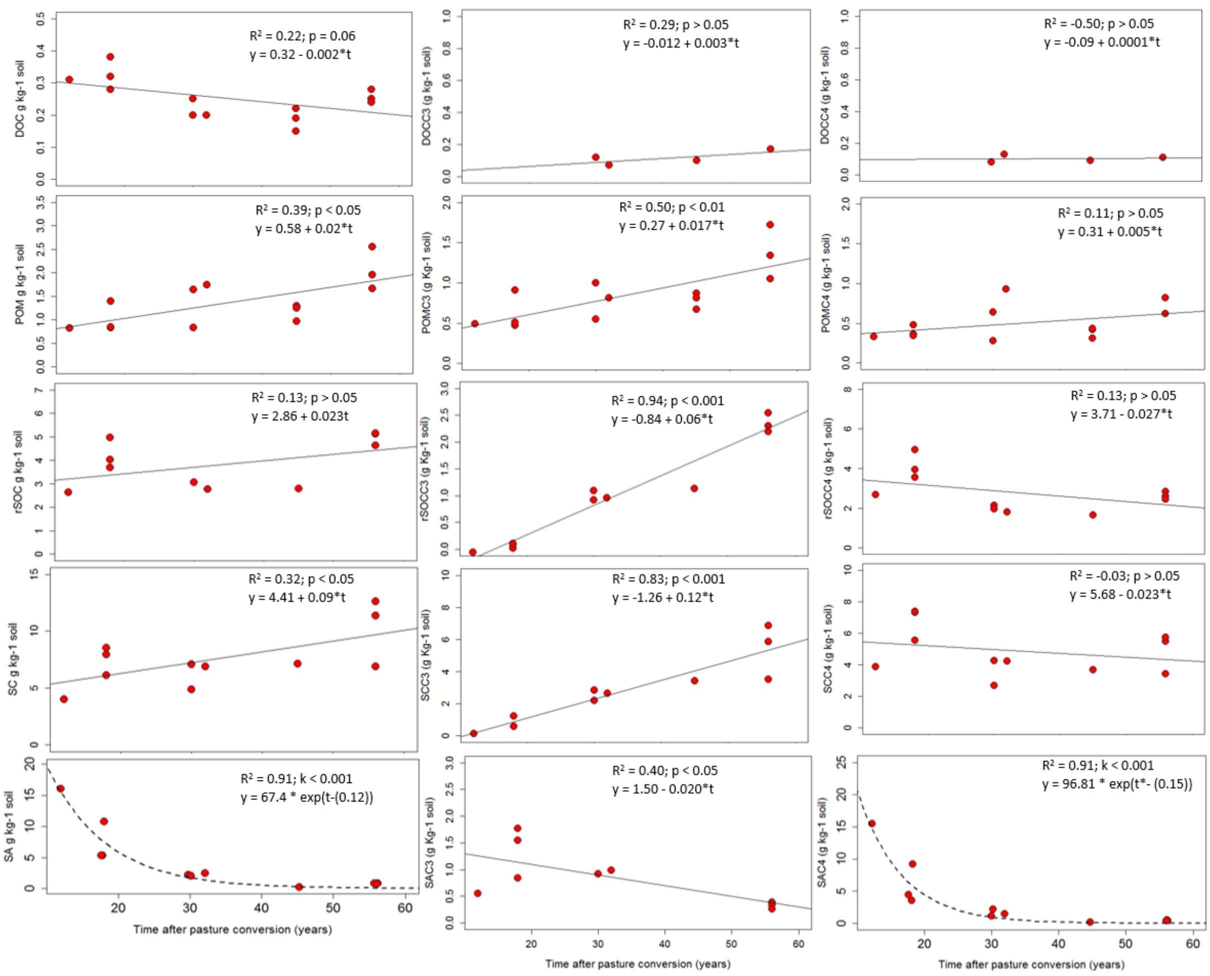


Figure 2. SOC fractions dynamics during OP cultivation time in the topsoil layer (0-10 cm). Black lines are the fitter linear or nonlinear regressions; the model and parameters are given as inserts. C concentration (left graphs), C3 (central graphs) and C4 (right graphs) in the different SOC fractions are illustrated.

The dynamics of C concentration in all fractions with OP cultivation time were best represented by linear models, except for S+A. A nonlinear exponential decay model described the dynamics of C concentration with time in S+A (Fig. 2). In this same fraction, C concentration exhibited the widest range of variation among all fractions and varied from 16.1 to 0.7 g kg^{-1} in an exponential decline fashion with a high exponential rate constant (k = 0.12; Fig. 2) that resulted in a short half-life time of 5.78 yrs. In contrast, POM and S+C dynamics over time were fitted by linear models with positive slopes which suggests no approach to a new equilibrium in the C concentration in these two fractions (Fig. 2). However, The C gain observed in POM and S+C did not compensate for the large loss of C in S+A.

5.4.3 Soil carbon content dynamics in OP- and pasture-derived fractions

Cultivation time of OP affected the C concentration of OP-derived C in nearly all the separated SOC fractions in this study. In contrast, pasture-derived C concentration was found to change only in S+A. OP-derived C concentration increased linearly over time in POM, rSOC and S+C. The S+C fraction showed the greatest increase of OP-derived C followed by rSOC and POM. Only in the DOC fraction, OP-derived C did not change over time, however this was most likely due to the few data points obtained for this regression analysis. S+A was the only fraction where OP-derived C decreased, but its loss rate was similar to the one of POM. On the other hand, the loss of pasture derived C in S+A was best fitted by an exponential decay with a very high rate constant value. In consequence, the half-life describing loss of pasture-derived C in this fraction was very short (4.6 yrs.). Pasture-derived C in all the other four isolated fractions did not change significantly over OP cultivation time, but the trends were inverse to OP-derived C for rSOC and S+C.

5.5 Discussion

The long-term time scale allowed us to detect different C dynamics during the first and second OP rotation cycles after pasture conversion. Over the entire period of OP cultivation and including the effect of LUC, we found a V-shape response to pasture conversion in the fractions POM, rSOC and S+C for C concentration, SOC distribution and mass contribution. This means that these SOC fractions responded negatively to pasture conversion into OP in the short-term of first cultivation cycle, but thereafter the trend reversed. This finding backs those in Quezada et. al (2019), where pasture conversion led to a reduction of bulk soil C concentration during the first 41 years of OP cultivation, followed by a partial recovery phase (Fig. S1). More specifically, SOC concentration in POM, S+C and rSOC decreased during the first OP cycle and then returned to similar levels as in pastures. Among these three fractions, POM showed the greatest decrease in SOC concentration. Indeed, almost half of the initial SOC was lost in this labile fraction. In consequence, POM was the most affected fraction by LUC during the first OP cycle, which partly agrees with our hypothesis that POM will be the most responsive fraction to pasture conversion, despite being the less important fraction on term of mass contribution. Also, the found V-shape pattern response of SOC in most fractions to LUC contradicts our expectation of an overall shift in the size and distribution of SOC pools.

Interestingly, this initial sharp decline of POM was followed by a strong recovery phase with an increase of 86% of C concentration of this fraction in the second OP cycle compared to the first OP cycle. The initial strong decline of POM is in line with numerous studies that have pointed to this fraction as one of the most sensitive to LUC and management because of no physical protection of this labile fraction (von Lützow et al. 2007; Miller et al. 2019; Poeplau and Don 2013; Yamashita et al. 2006). However, to our knowledge only few studies in other agroecosystem types (Anaya and Huber-Sannwald 2015) and none in LUC to OP, so far, have tracked the fate of POM over decadal time scales (more than 30 years) and demonstrated empirically the recovery of this labile fraction over long-lasting cultivation. This reversal pattern, can be explained, at least partly, by the linear increase of OP-derived POM that made 61.4% of total POM across the first OP cycle and 63.8% across the second OP cycle, while pasture-derived POM remained unchanged over the entire OP cultivation time. Such no change in pasture-derived POM suggests a low pasture C mineralization rate, which can be due to the inherent resistant chemical composition of pasture derived OM, i.e. highly lignified root debris (Marín-Spiotta et al. 2008). Alongside with the decrease of labile C in POM after LUC, the SOC bounded to S+C and rSOC decreased as well. This is somehow an unexpected short-term outcome given the supposed high degree of stabilization of SOC in the inert rSOC and mineral S+C fractions. The rSOC and S+C fractions consisted of 11.9 and 19.9% OP-derived SOC, respectively, across the first OP cultivation cycle, which suggest that these fractions are not inert but active. Similar findings were reported by Poeplau and Don (2014) in LUC from C3 crops to *Mischanthus* (C4 vegetation), thus the apparent high chemical resistance of SOC in this fraction to decomposition is not as though.

In partial agreement with our first hypothesis pastures conversion into OP depleted the soil δ^{13}C signature of all measured fractions, but changes over time were mostly linear. It is noteworthy that the accumulation of new OP-derived C in POM remained rather weak, with a small regression slope as compared those of SOC and S+C fraction across the first and second OP cycles, suggesting a possible saturation of C in POM. In line with this, recent studies have demonstrated (Quezada et al. 2019; Rüegg et al. 2019) the inefficiency of aboveground C inputs in OP plantations, i.e. from accumulated palm fronds, to become stabilized in SOC, which could explain the poor increase of OP-derived C in POM. We expect that if better management strategies would be used to incorporate the decaying OM of first-cycle plantations, the overall soil C storage would be further enhanced by an increase of POM OP-derived C accumulation. Another potential important factor to explain the V-shape pattern found in the C concentration in rSOC and S+C is the large input of above and belowground C during the initial years of plantations renovation when the first OP rotation cycle ends. During this plantations' renovation phase the aboveground biomass of the old palm trees fall progressively and start to decay over a 5 to 6 years' time period. From our finding of increased OP-derived C in rSOC from 11.9% in the first OP cycle to 43.3% in the second OP cycle and in the case of S+C from 19.9% to 49.8%, we can infer that decaying OP-derived C during the plantations' renovation phase is stabilized mainly as SOC into rSOC and S+C. Considering that these two fractions together constitute a major contributor to the C mass, this shows the importance of a proper management of the plantations' renovation for sustaining a long-term SOC storage.

Pasture conversion into OP affected the distribution of SOC in the POM, S+C and S+A fractions. Interestingly, the dynamics of the distribution of total SOC in these fractions generally mirrored the shift in the fractions' mass distribution. This suggests that a simplified fractionation scheme where only POM, S+C and S+A fractions are separated could provide useful insights into the dynamics of SOC. This simple fractionation should involve only density separation of POM after disruption of aggregates by weak sonication and physical separation by wet sieving of S+A and S+C fractions. Although not significant ($p = 0.07$), the greatest change in SOC concentration between pasture, OP cycle 1 and 2 among all separated fractions was found in S+A. For this fraction, second-cycle plantations had only a fifth of the initial C concentration in pastures. S+A also displayed the fastest decay rate among all fractions with a very short half-life time of only 5.8 years. The decline found in both pasture- and OP derived C in this fraction explain well this very fast turnover rate.

In summary, we have found that over the long-term pasture conversion into OP could be of relevance in the context of enhanced soil C sequestration. Similar conclusions were made for, soil fertility and physical condition (Quezada et al. 2020 unpublished). This is because in terms of C sequestration it is preferable to store C in more resistant SOC fractions like S+C and rSOC, while for soil fertility storing of C into more labile C fractions such as POM is preferred (Chenu et al. 2019). Thus, we can conclude that in the long-term, as we demonstrated with two consecutive cultivation cycles, not only C sequestration, but also soil fertility are benefited from the conversion of pastures into OP. Also, we can ascertain that the time span considered for the assessment of LUC into OP can strongly impact the conclusions that might be draw both in terms of C distribution of SOC into different fractions and patterns of SOC dynamics. The observed recovery phase in POM, rSOC and S+C is of yet unknown duration and this highlights the need to either monitor SOC changes beyond two OP rotation cycles or alternatively employ soil C models to predict future changes.

6 General Discussion and Perspectives

*“**Ecuadorians** are strange and **unique beings**, they **sleep peacefully** amid smoking **volcanoes**, they live in **poverty** amid incomparable **wealth** and they **cheer up** with **sad music**”*
Alexander Von Humboldt

Crossing Meta River in Los Llanos region of Colombia

6.1 Major findings of this work

The overall aim of this thesis was to quantitatively assess the impacts of deforestation-free alternatives on soil biogeochemical properties and ecosystem C storage. In the foregoing manuscripts of this thesis (1 and 2), I have discussed and presented evidences that support the first general hypothesis of this research work that deforestation-free OP expansion in the tropics can substantially reduce ecosystem C losses compared to LUC with forest clearance. My findings also confirmed the second general hypothesis that changes in SOC storage in pastures and savanna derived OP plantations vary depending on the inherent characteristics of old C4-C, i.e. with respect to soil clay content. Importantly, I also showed that improved management practices can substantially reduce ecosystem C losses and even increase soil C storage and functionality (Manuscript 2 and 3), which fits the third general hypotheses. In this chapter, I aim to synthesize and integrate the main findings of the presented manuscripts to present a perspective for a more sustainable OP expansion in the tropics. Finally, I also aim to present a perspective with some topics for future research directions and some more speculative ideas that could be of future interest.

Besides assessing the impacts of OP cultivation across time-scales by using one-cycle and two-cycle OP chronosequences, I also took advantage of the variations in the ^{13}C isotopic signatures of organic C in soil in all the four studies of this work. Such changes in the soil's ^{13}C isotopic distribution revealed a few of the mechanisms that stabilized/decomposed SOC (Manuscripts 3 and 4). The extended time periods used in the two chronosequences of this thesis are of high relevance because at present most of the available studies on the impacts of OP cultivation on SOC storage are too short term or just focused on plantations of specific age, i.e. mature plantations of around 8-25 years old. Moreover, various studies have highlighted the need for long-term assessments of changes in SOC and soil properties. Indeed, the absence of data regarding the time required to reach a new equilibrium after LUC into OP agriculture could very likely be due to the lack of long-term studies (Dislich et al. 2016). Here I filled such gaps in two alternatives and important ongoing LUC types, pastures and savanna conversion into OP plantations.

I demonstrated that conversion of pastures into OP has important implications for C storage in soils. Moreover, due to the extensive area covered by pastures in the tropics, about 2.5 million ha in Los Llanos of Colombia and 60 million ha in Brazilian Cerrado, of which around 18 million ha are degraded (Guimarães et al. 2004), and their suitability to be replaced by OP agriculture, it is crucial to determine the effects of this LUC in order to provide scientific-based evidence that could inform well the direction of OP future expansion at regional scales. Another important topic covered by this thesis work is the quantification of important SOC dynamics metrics. This is somewhat surprising because although a big body of scientific work has been focused on the spread of OP plantations, the rates of change and time length of the decreasing and stabilization phases in SOC dynamics determined in this work, empirically, are to the best of my knowledge for first time reported. This is also noteworthy, because despite the great concerns to reduce the threats of OP expansion at the expense of deforestation and the existence of mounting non-natural sciences related literature (i.e. modeling studies and policy papers), to date empirical field-based evidences associated with the impacts of alternative deforestation-free LUC trajectories for more sustainable OP expansion is lacking.

This thesis contributed to new insights regarding changes in biogeochemical aspects of one- and two-cycles of OP cultivation after pastures and savanna conversion. Major findings of this work can be summarized as follows.

Two-cycles chronosequence (long-term impacts following pasture conversion)

- Pasture conversion into OP is neutral from an ecosystem C storage perspective. This finding shows that this LUC has a potential to mitigate greenhouse gas emissions as compared to the dominant practice of cultivation on previously deforested land.
- Gains in OP biomass were offset by SOC losses that occurred mainly during the first OP rotation cycle. During this first cycle, the loss rates of pasture-derived C exceeded the accumulation rates of oil palm derived C and this pattern explained the overall decrease of soil C stocks.
- Converting pastures with high initial SOC stocks will lead to high losses following LUC to OP. Thus, abandoned pastures with reduced C stocks are better suited for OP cultivation.
- Soil C stocks reached a new equilibrium during the second plantation cycle following the initial sharp reduction phase.
- The overall no reversal trends (initial decrease with subsequent stabilization) in SOC stocks with extended period of SOC loss confirms the gradual and slow response of SOC to LUC and management, which highlights a great need to assess changes in soil C dynamics over long timescales, i.e. OP land use for more than 30 years (beyond one rotation cycle).
- Subsoil C stocks (30-50 cm) were affected along the 56 years of OP cultivation.
- There is a tradeoff between soil chemical properties and SOC. However, in the present management scheme, soil nutrient supply capacity relies mostly on the frequent application of nutrients via mineral fertilizers in OP plantations and less on nutrients release from SOM mineralization.
- Soil chemical fertility was maintained or enhanced, indicating the sustainability of OP cultivation on the long-term and its reversibility to the initial or a new land use.
- From the observed recovery of topsoil C content and slower C dynamics in beneath surface layers a possible recovery of original SOC stocks levels could eventually be reached in a third OP rotation cycle. This pattern is partly consistent with the common U-shaped trend (Covington curve) reported in other long-term SOC dynamics studies (Covington 2011; Zak et al. 2011).
- Evidence from fractions composing SOC support the recovering pattern found for bulk soil C content. C concentration in both labile (POM) and more stable fractions (S+C and rSOC) returned to similar levels as in pastures. This is of relevance in terms of soil C sequestration and

soil fertility. Importantly, such recovery of C in different SOC fractions is yet of unknown duration, which highlights the potential of pastures conversion into OP to become important C sinks as opposed to deforestation.

One-cycle chronosequence (savanna conversion)

- Savanna conversion into OP resulted in a positive ecosystem C outcome. This finding shows that this LUC has a potential to mitigate greenhouse gas emissions as compared to the dominant practice of cultivation after deforestation.
- Soil C stocks were not affected due to savanna conversion into OP. This was mainly explained by the slow decomposition of savanna-derived C and effective oil palm-derived C accumulation that offsets the small savanna C losses.
- Management practices within plantations (management zones) largely outweighed the importance of LUC to explain changes in soil biogeochemical properties.
- Soil biological activity was maintained or even enhanced, when organic amendments (i.e. crop residues from frond piles) were applied. In contrast, heavy mineral fertilizers reliance led to reduced biological activity.
- Changes in SOC stocks within plantations are mainly driven by C inputs via OP fine roots. Fine OP roots were directly affected by nutrients addition and drive changes in soil microbial properties. Therefore, practices seeking to stimulate root development, i.e. nutrients addition, would increase C storage capacity in savanna OP cultivated areas.
- If the benefits of increased SOC stocks, enhanced fertility and soil microbial activity observed under receiving input areas (i.e. crop residues and/or fertilizers) in plantations are scaled-up to the whole plantation level, the potential of savanna conversion as an important long-term C sink could be further increased.
- Producing countries should create policies and development agendas that encompass incentives to cultivate OP in savanna areas preferably in unproductive pastures so as to preserve as much as possible unique savanna ecosystems.

6.2 Perspectives

Insights gained into the dynamics of SOC after savanna and pastures LUC were presented before. However, as OP expansion in the tropics has been identified as a primary driver of deforestation, particularly in Southeast Asian countries (Fitzherbert et al. 2008; Gaveau et al. 2016), there is mounting evidence on the effects of this LUC on various important ecosystem services. This has resulted in a lack of empirical evidence regarding soil changes associated with alternative LUC trajectories (i.e. plantations derived from cultivated land or pasture areas) for OP expansion. Therefore, future challenges remain for the understanding of the mechanisms underlying SOC dynamics in deforestation-free scenarios for OP development and other important neglected biogeochemical aspects. Research efforts should continue in savannas regions and should include research themes like:

- Studies on tropical LUC have demonstrated that time after vegetation change is a predominant factor to determine the magnitude and direction of changes in SOC stocks (Bárcena et al. 2014; Guo and Gifford 2002). However, a key management practice in OP cultivation is plantation renovation and this has been, so far, a neglected aspect. Plantation renovation usually occurs 25-30 years after plantation establishment, when harvesting of fruit bunches starts to be difficult due to palms heights and poor productivity. When plantations are renovated, soil tillage might or might not be performed. Then, the vegetation of the old OP plantation progressively decays in the field after injection of an herbicide, i.e. glyphosate, to the palms' trunks. As a consequence, a high pulse of OM from above and belowground decaying biomass enters the soil during the first 3-4 years following renovation. Whether most C inputs from decaying OM is mineralized to CO_2 or stabilized as SOC is still unknown. Also, potentially useful management practices for the decaying OM, i.e. incorporation by mean of reduced plowing, should be evaluated so that the greater SOC stabilization could be attained after plantation renovation.
- More in-depth research regarding the relative contribution to SOC of aboveground and belowground OP plant material (root *vs.* shoot) is also needed. Even though in Manuscript 2 and 3 it was demonstrated that fronds accumulation is not an effective practice for SOC stabilization, a better quantification of annual C inputs over long time periods, i.e. one full rotation cycle, is needed for a more accurate estimation of the contribution of other important C inputs like OP roots to SOC. Also, studies on management practices available to enhance the efficacy of pruned OP fronds for to increasing SOC stocks and other biogeochemical aspects are scarce. Consequently, effects of belowground inputs and better management practices for aboveground inputs remain to be studied.
- Iron and aluminum oxides contents along with clay type and content are known to be key drivers of soil C cycling in tropical soils (Six et al. 2002). Indeed, the effect of mineralogy on soil C stability is well documented. Nonetheless, in the case of OP cultivation, not much emphasis have been given to investigate the role of soil mineralogy on the directions and strength of SOC dynamics.
- Predictability of future changes in SOC storage in OP plantation after multiple cycles (beyond two cycles) by mean of SOC models like the Rothamsted C model (RothC) remains to be studied. Even though the RothC model was developed for soils of temperate climates, it has been applied to a wide range of climate conditions. Also, to the best of my knowledge, the performance of the RothC model has not been evaluated in simulating SOC storage in OP plantations. Thus, a validation procedure with the ^{13}C and fractionation data produced in this thesis could be performed. The combined use of ^{13}C and fractions data can also evaluate the dynamics of the RothC fractions and not only the bulk SOC dynamics. It is also noteworthy, that the fractionation procedure performed for Manuscript 4 presents the advantage that the

isolated fractions correspond to the conceptual SOM pools in the RothC model (Miller et al. 2019).

- Although our conclusions rely on the quantification of the most important C pools in agricultural ecosystems, challenges remain, i.e. data collection at better resolution, considering the various types of pastures and their soils, to confirm that the cultivation of pastures entails a positive ecosystem C balance and reduce effectively the ecological footprint from OP expansion.
- Estimate SOC sequestration potential and assessing how far converted savanna or pasture soils are from their C saturation level is another topic for future investigation. For example, the SOC saturation deficit or sequestration potential can be estimated as the difference between SOC saturation and SOC stored in the fine fraction (< 20 um), where the SOC saturation could be obtained by using the proportion of the fine fraction in the equation proposed by Hassink (1997) in (Chen et al. 2019):
  SOC sat = 4.09 + 0.37*fine fraction
- Investigate possible shifts in soil-food webs. The microbial community structure/composition (fungal:bacteria) have shown to be affected by OP cultivation (Kerfahi et al. 2014; Lee-Cruz et al. 2013; Tripathi et al. 2016). However, virtually all studies have been focused on forest to OP conversion, with no emphasis on alternatives LUC to OP like the ones covered in this thesis work. Furthermore, to my knowledge, none of them studied microbial community composition changes considering samples from the different MZs in OP plantations. Therefore, an improved understanding on how both alternative LUC to OP and management practices affect soil microorganisms is critical to generate novel insights into key C cycling aspects controlled by soil microbes like C mineralization and accumulation rates. Phospholipid fatty acids (PLFA) and DNA sequencing analyses can be used to characterize soil microbial communities. However, to conduct these analyses it will be important to store samples at -20°C and this could be challenging given the remote field locations.
- Increasing breeding efforts to develop more productive OP varieties with more abundant and rapid growth root system are needed. Although new OP hybrids have been developed and are already in commercial scale use, the focus of their development has been on better resistance to the fatal bud rot disease that has affected extensive OP areas in South America. Future breeding efforts should also focus on traits that aim to increase the ability of OP to adapt to climate change (most likely increasing drought) by developing more aggressive root system which, in turn, will also increase belowground C inputs and nutrient use efficiency.
- In addition to the ecosystem C storage benefit when OP replaces pastures, recent studies on biodiversity in the Neotropics have also demonstrated that OP plantations can support greater biodiversity than sawn pastures (Prescott et al. 2016) and that converting pastures into OP will have a low impact on endemic and threatened vertebrates (Ocampo-Peñuela et al. 2018), provided that some spatial distribution patterns are applied at landscape scale. Such positive

impact of pastures conversion on biodiversity should be tested at greater scales and on more animal and plant taxonomic groups.

6.2.1 On future sustainable OP development

There are several reasons to be optimistic about the prospects of sustainable OP production. More than fifty years, still underway, of OP cultivation in non-forested areas in Colombia and other Latin American countries clearly shows that alarming C emissions from forest conversion can be curtailed. Even though savannas areas do not exist in SE Asia OP producing countries, extensive areas of grasslands amounting about 9 million ha (Syahrinudin, 2009) containing mainly *Imperata cylindrica* grass exist there. These grasslands and other degraded land areas set an opportunity for the expansion of deforestation-free OP agriculture. On the other hand, several studies including Manuscripts 2 and 3 have demonstrated the efficacy of different management practices, i.e. compost, cover crops and intercropping, to increase soil fertility and biodiversity in OP agroecosystems. In this context, while challenges remain regarding the adoption of improved management practices, the progress made by OP farmers and biophysical studies, as well as the increasing awareness of multiple stakeholders in all levels regarding environmental conservation (i.e. certification of OP) set the stage for a pathway toward sustainability in the eminent expansion scenario of OP plantations.

To build on the achievement of enhanced soil chemical fertility in main OP producing countries, which contrasts sharply with the general high fertility depletion of African soils, it is paramount to start adding organic inputs to OP cultivated soils. Only organic inputs will help boosting microbial activity, increase SOC and enhance soil physical properties like structure and soil moisture retention. In contrast with the current standard management strategy across main OP producing countries, i.e. Colombia and Indonesia, with well-defined MZs, the strategy I propose here (also in Manuscript 2) suggests a paradigm shift. This paradigm transition encompasses a gradual change in nutrient inputs type from predominantly mineral to organic inputs and towards more biologically oriented processes so that a wide range of soil functions including microbial activity, SOC sequestration, N fixation and GHG emissions can be enhanced and not only chemical fertility.

In Manuscript 2, I suggest a focalized restoration of natural vegetation at the IZ areas (every second between palm row). In parallel with such understory restoration of natural vegetation, modifications in the timing, placement and integration of nutrients and OM sources should be aimed. All together could promote greater accumulation of more stabilized SOC pools by harnessing biological processes that could concomitantly increase short-term biological soil functioning and fertility and should be adopted in the remaining surface area of plantations (zones F, H and W). Periodical movement of the most decomposed palm leaves fragments from the frond pile (F) to the circled area around the palm trunks (W) could be part of this combined long-term-short-term management strategy. This partial displacement of decomposed OM could increase plant-available nutrients and boost soil microbial activity in the OP rooting zone and therefore reduce the need of external inputs, without affecting the high microbial functioning and OP root development at F. Also, the partial displacement of decomposing fronds should be synchronized with the peak of the nutrients demand phase, so that the nutrients availability coincides with crop requirements and losses are minimized.

Another important source of organic C inputs could be the compost derived from harvested fruit bunches, which have proved to be effective in increasing plant available nutrients (Pauli et al. 2014). Importantly, mineral fertilization should continue to contribute to overall soil chemical fertility, but organic matter inputs must play the central role in the maintenance of productivity in OP cultivation. Although, achieving such complex combination of short-term and long-term management goals is challenging and ambitious from a practical standpoint, future OP cultivation systems must strive to reduce the excessive use of synthetic fertilizer and pesticides by embracing more ecologically oriented practices to increase its productivity and overall sustainability. In addition to the management approaches noted before in this section, legume intercropping is another highly recommended practice not only to increase soil C inputs and increase SOC stocks, but also to enhance the overall soil functionality. In fact, in Colombia I attested the use of various legume cover crops such as kudzu (*Pueraria Phaseoloides*), Mucuna (*Mucuna bracteata*), Desmodium (*Desmodium heterocarpon),* Canavalia (*Canavalia brasiliensis)* and others. These leguminous cover crops are well adapted to the local conditions and agronomist and practitioners say that this management practice works well and helps to control weeds, maintain soil moisture and increase OP root development.

The development and implementation of all the described management options necessitates a systematic framework to assess at different scales their outcomes in terms of productivity and environmental sustainability. To do so, I envisage coordinated on-farm research via private-public collaboration as a feasible option. In this sense, participatory research approaches, i.e. Companion Modelling (ComMod) as in the OPAL project, for agroecology experimentation with close participation of OP farmers, local research institution and local governments should be promoted. This collaborative research efforts aiming scientific and practical impacts could provide an effective way to advance sustainable OP production and should facilitate the understanding of complex, socio-environmental systems. Agroecological research topics of common interest in this public-private partnership should pursue short-term and long-term productivity and sustainability goals. As priorities differed by country, in countries like Colombia or Indonesia where agricultural yields are fairly good, more attention should be given to outputs that go beyond agricultural aspects. For instance, the effects of OP cultivation on biodiversity, water and human well-being.

A relatively recent feature found in tropical grasses and particularly for those in the genus, *Brachiaria spp* is their capacity to suppress soil nitrification through the release of organic root exudates. This plant-soil trait named “biological nitrification inhibition” (BNI) is being investigated at the field and laboratory scale, and the underlying mechanisms driving this process have also being well described elsewhere (Subbarao et al., 2009). Up until now, much emphasis has been given to the assessment of BNI in pasture lands transformed into annual cropping systems (i.e. corn and wheat). Experimental results indicate multiple beneficial effects of BNI on subsequent crop N use efficiency, yield capacity and nitrous oxide (N_2O) emissions rates (Moreta et al., 2014). Nonetheless, BNI is still an incipient research topic and its effects and effectiveness for different land uses and cropping systems like perennial crops remains largely unknown and it constitutes a subject of future investigation.

BNI offers a promising alternative to cope with the negative effects of excessive N fertilizers addition. Moreover, N management strategies that seek to reduce N use and improved crops N use efficiency are desirable in the context of improving the sustainability of PO production. Fertilizer recovery efficiency is low in OP systems due to leaching and gaseous losses of nutrients (Gerendás et al., 2014). Such losses also impact profitability. Therefore, suppression of soil nitrification by the presence of natural nitrification inhibitors and increase N use efficiency will be of economic and environmental benefit for OP growers and surrounding communities. Since most OP plantations in Los Llanos have been established either on previous savanna or pasture areas, there is a potential BNI legacy effect that can be influencing N cycling in these soils. In this thesis work I did a preliminary evaluation of the BNI legacy effect in OP plantations derived from former pasture and savanna areas (Appendix 1). This work was carried out in collaboration with the Tropical Forages group at the International Center for Tropical Agriculture CIAT, Colombia, which is one of the worldwide leader groups working on BNI. In my opinion, from the preliminary results in Appendix 1, promising work in this direction could be done. For instance, monitoring of N_2O emissions *in-situ* and also in laboratory incubations could be performed.

Both the effects of cover crops and BNI on C and nutrients dynamics are worthy of investigation. The use of either BNI or cover crops strategies separated give rise to different OP-cropping systems. On one hand, establishing OP in companion with shade-tolerant legume cover crops can be seen as OP-cover crop system and on the other hand integrating OP with pasture production of BNI capacity can be seen as an OP-livestock system. These two cropping systems could serve as a model for sustainable intensification of PO production in other regions. As a matter of fact, in Los Llanos of Colombia, these two cropping systems are being well adopted, however their effects on soil biogeochemical aspects remain almost fully unknown. Figure 1 in this chapter, provides a conceptual model that could serve as a framework for future research work on N cycling aspects in the Legume-OP System and Livestock-OP integrated system.

Finally, and although it has been noted in Manuscripts 1 and 2, I emphasize here that I do not advocate or recommend future expansion of agriculture in savanna ecosystems because these are valuable ecosystems worth to be preserved for their natural value. Hence, I warn cautiousness with the interpretations of my findings so that they are not used to promote savanna conversion. Also, because all my study sites were located on flat not-flooded topography areas and far from gallery forest areas, the obtained outcomes should not be generalized to other OP growing areas with hilly topography and nearby gallery forests.

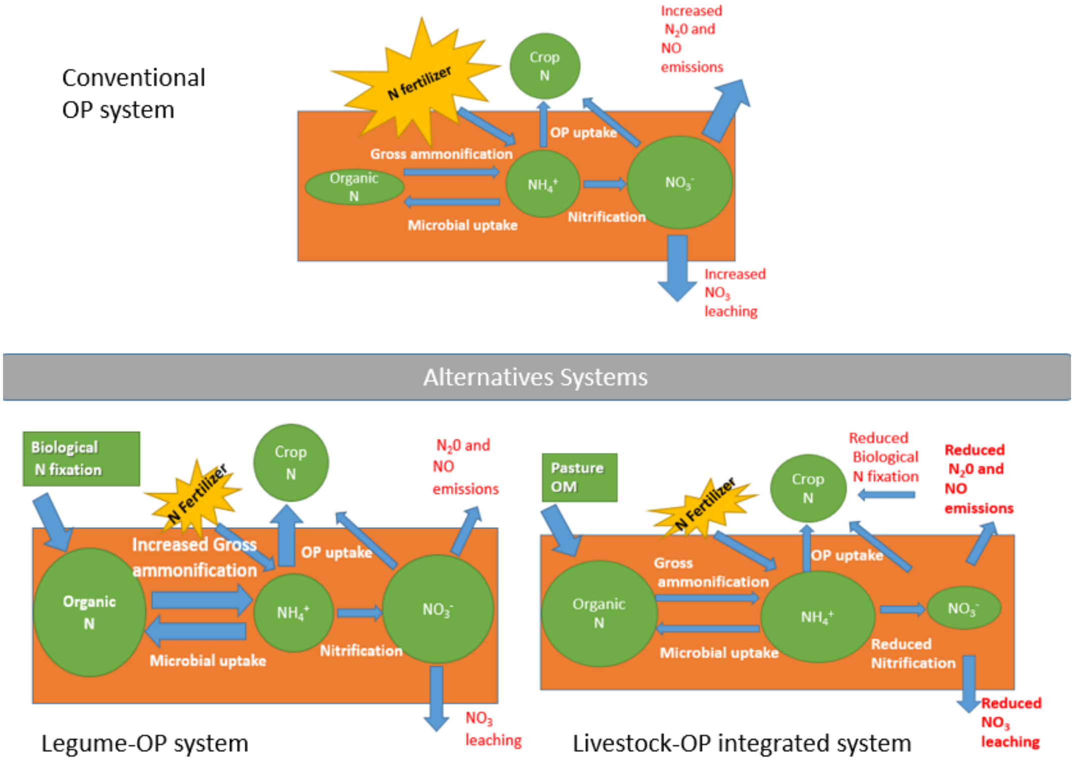


Figure 1. Conceptual diagrams of N cycling dynamics in OP plantations established on previous pastures areas. Upper figure: conventional sole OP system where the inorganic N pool is dominated by NO_3 due to high nitrification and heavy use of N fertilizers. Mineralization of SON is increased due to LUC resulting in high rates of gross ammonification. N loss processes such as gaseous emissions and NO_3 leaching are characteristic of this cropping system. Lower-left figure: incorporation of legumes into OP plantations provides organic N inputs that increases the SOM labile fraction. Soil N turnover as expressed by rates of gross ammonification increases and therefore levels of plant available N are maintained. Lower right figure: the incorporation of *Brachiaria* pastures into OP plantations will enable control on nitrification, thus the inorganic N pool will be composed by NH_4 predominantly. The dominance of NH4 over NO3 in this system will reduced the vulnerability of N to be lost.

6.2.2 On speculative ideas

One of the fundamental questions regarding OP sustainability is whether it would be possible or not to continue the use of land once OP cultivation ceased. From the evidence presented here, land cultivated with OP in former savanna areas can be used either for other agricultural crops or patches of forest vegetation could grow in case plantations are abandoned (Fig. 2). In the case of abandoned OP plantations that could allow successive tree vegetation development, it is known that fire suppression and savannas nutrient enrichment are two fundamental conditions that can result in increasing tree cover on natural nutrient-poor savanna areas (Parr et al. 2014; Silva et al. 2013). From our findings and observations, I know that these two conditions are met with OP cultivation in savanna regions. Meanwhile, if natural vegetation is allowed to grow during the timeframe of OP cultivation on the IZ area, as I recommend in Manuscript 2, this could allow the progressive development of forest tree species, while suppressing savanna grasses, which would lead to tree encroachment after various OP cycles, i.e. three to four, and ultimately to a progressive transition of savannas into an early stage of forest succession. Further, various developing countries with important savanna areas, i.e. Colombia and Brazil, do not prioritize savannas conservation in their development programs. Consequently, there are incentives to continue the expansion of agriculture on savannas. Nonetheless, if OP plantations are abandoned, in the long-term, owing to different circumstances, it is possible that forest patches will cover important extensions of savannas instead of OP plantations. The overall outcomes of such forest-savanna mosaic landscapes, however, can be perceived as ambiguous featuring not only benefits but also tradeoffs. On one hand, as a matter of fact, savannas biomes are currently being lost at unprecedented rates in some parts as Brazilian Cerrado and Los Llanos of Colombia (Batlle-Bayer et al. 2010; Romero-Ruiz et al. 2010) and further conversion to any other land use, including forest, will be detrimental for a host of ecosystem services provided by savannas. Among the threats to savanna conversion are loss of high endemic biodiversity and alteration of hydrological and nutrient cycles at regional or even global scales (Abreu et al. 2017). On the other hand, increasing forested areas worldwide have been increasingly seen as a promising mean to respond effectively to major environmental hurdles such as global warming (Bastin et al. 2019), however this is also a topic of great debate.

In this same context of land use after OP cultivation and from an agronomical perspective cultivating land with important cash crops should not be burdensome to implement. For example, the current development of agriculture in savanna regions like in Brazil and Colombia include soybean and maize production for which important technological advancements to increase productivity and reduce the use of external inputs, i.e. pesticides, have occurred. In case this cash crop scenario is developed after OP cultivation, a suite of management plans and policies should be employed to reduce the risk of high environmental impacts in savanna regions due to agricultural intensification.

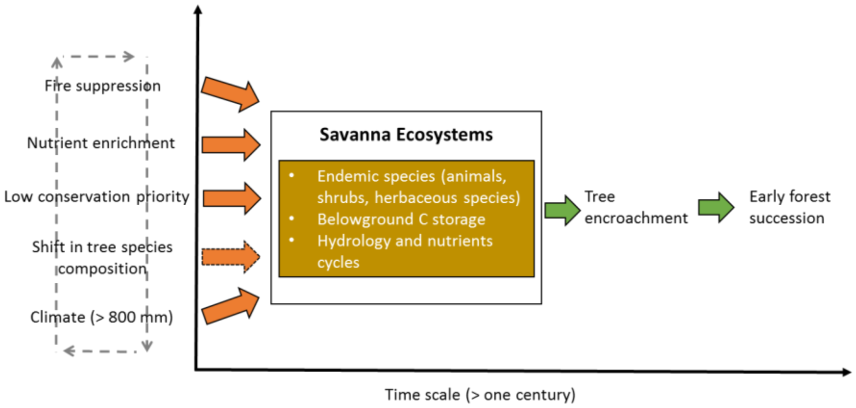


Figure 2. Conceptual diagram of increasing tree cover in savanna regions after OP plantations abandonment

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*“**Genius** is **one** percent **inspiration** and **ninety-nine** percent **perspiration**”*

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8 Supplementary information (of each Chapter)

“Two ***roads diverged*** *in a wood and I* **-** *I* ***took*** *the one* ***less traveled*** *by, and that has* ***made*** *all the* ***difference****”*

Robert Frost

8.1 Supplemental Material Manuscript 1

Figure S1. Map of study sites and potential area for expansion of oil palm in pasture lands in the Neotropics.

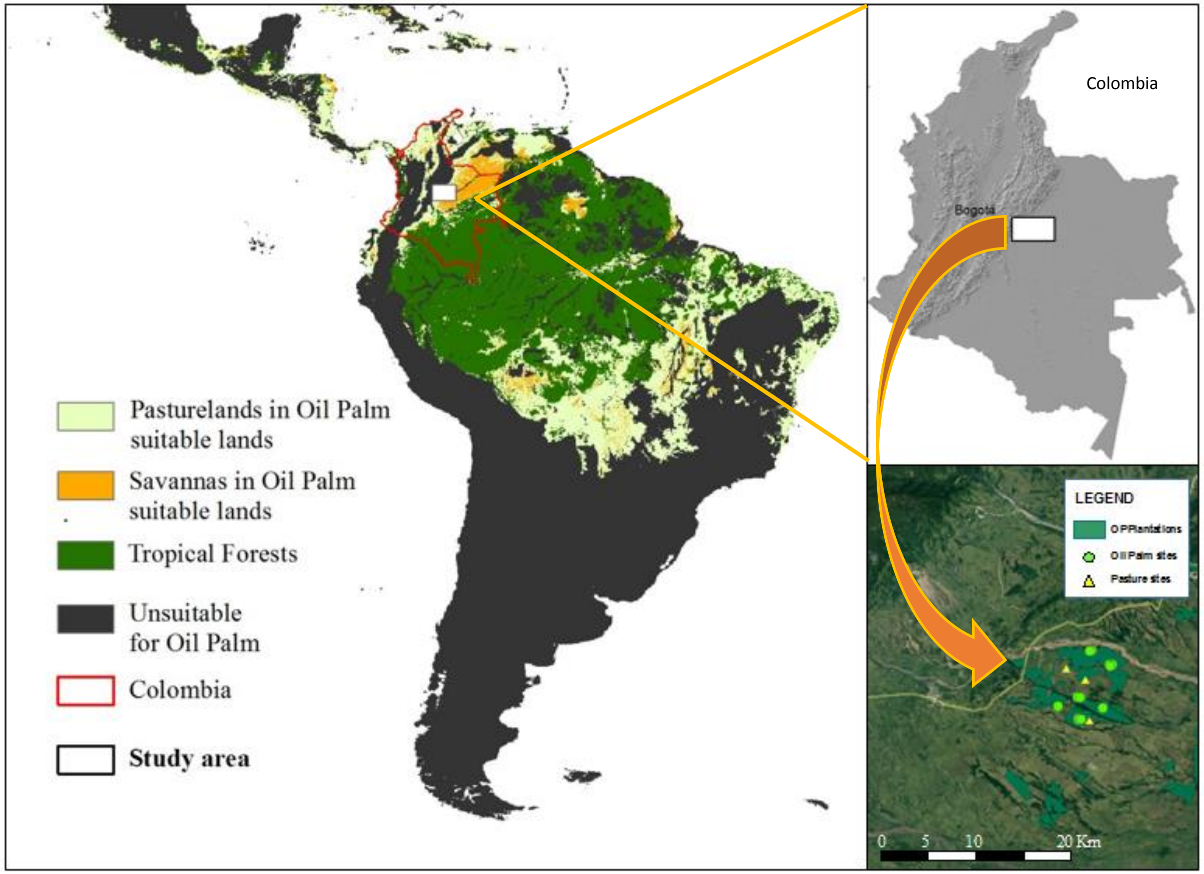

Table S1. Set of models tested for the bulk soil and pasture- and oil palm-derived carbon.

Soil layer	Model type	Function	R2	P value of model	P value of age	AIC	k1 (yr-1)	e/x1/x2/A/c[a]	k2 (yr-1)	Slope 1	Slope 2
Bulk soil; 0-50 cm	Linear	F(t) = 99.64 - 0.78*t	0.66	***	***	126.5	-	-	-	-	-
Bulk soil; 0-50 cm	Segmented	F(t) = 105.71 - 1.26*t; F2(t) = 0.18*t: BP:36.1	0.73	-	***	124.3	-	-	-	***	NS
Bulk soil; 0-50 cm	Monoexponential	F(t) = 47.77 + (104.65-47.77)*exp(t*- (0.028))	0.73	-	-	125.8	NS	NS (0.0533)	-	-	-
Bulk soil; 0-50 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
Bulk soil; 0-50 cm	Single exponential decay	F(t) = 102.2*exp(t*- (0.010))	0.71	-	-	124.9	***	***	-	-	-
OP-derived C; 0-50 cm	Linear	F(t) = 1.69 + 0.52*t	0.84	***	***	97.3	-	-	-	-	-
Pasture-derived C; 0-50 cm	Linear	F(t) = 93.6 - 1.19*t	0.80	***	***	129.2	-	-	-	-	-
Pasture-derived C; 0-50 cm	Monoexponential	F(t) = -32.59 + (396.96-(-32.59))*exp(t*- (0.013))	0.82	-	-	130.3	NS	NS	-	-	-
Pasture-derived C; 0-50 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
Pasture-derived C; 0-50 cm	Single exponential decay	F(t) = 98.1 * exp(t*- (0.020))	0.82	-	-	128.6	***	***	-	-	-
Bulk soil; 0-10 cm	Linear	F(t) = 28.9 - 0.19*t	0.40	**	**	97.2	-	-	-	-	-
Bulk soil; 0-10 cm	Segmented	F(t) = 31.9 - 0.42*t......	0.69	-	***	88.3	-	-	-	***	NS
Bulk soil; 0-10 cm	Monoexponential	F(t) = 19.4 + (32.05-19.4)*exp(t*- (0.066))	0.63	-	-	92.7	NS	***	-	-	-
Bulk soil; 0-10 cm	Biexponential	F(t) = 32 * exp (t*- (0.017) + 0.009 * exp(t- (-0.12)	0.74	-	-	88.8	**	***/NS	NS	-	-
OP-derived C; 0-10 cm	Linear	F(t) = 2.15 + 0.25*t	0.84	***	***	74.2	-	-	-	-	-
OP-derived C; 0-10 cm	Exponential rise to equilibrium	F(t) = -0.62 * exp (-0.038 * t) + 0.62)/0.038	0.91	-	-	66.0	**	***	-	-	-
Pasture-derived C; 0-10 cm	Linear	F(t) = 26.7- 0.44*t	0.75	***	***	101.2	-	-	-	-	-
Pasture-derived C; 0-10 cm	Monoexponential	F(t) = 4.37 + (32.01-4.37)*exp(t*- (0.054))	0.92	-	-	85.4	**	NS (0.07)	-	-	-
Pasture-derived C; 0-10 cm	Biexponential	F(t) = 31.95 * exp (t*- (0.042) + 0.008 * exp(t- (-0.10)	0.93	-	-	85.8	***	***/NS	NS	-	-
Pasture-derived C; 0-10 cm	Single exponential decay	F(t) = 31.4 * exp(t*- (0.037))	0.91	-	-	86.2	***	***	-	-	-
Bulk soil; 10-20 cm	Linear	F(t) = 22.20 - 0.18*t	0.55	***	***	86.2	-	-	-	-	-
Bulk soil; 10-20 cm	Segmented	F(t) = 24.49 - 0.36*t......	0.79	-	***	75.5	-	-	-	***	NS
Bulk soil; 10-20 cm	Monoexponential	F(t) = 12.6 + (24.28-12.6)*exp(t*- (0.048))	0.71	-	-	82.4	NS	***	-	-	-
Bulk soil; 10-20 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
OP-derived C; 10-20 cm	Linear	F(t) = -0.27 + 0.10*t	0.84	***	***	46.4	-	-	-	-	-
Pasture-derived C; 10-20 cm	Linear	F(t) = 22.5 - 0.28*t	0.77	***	***	85.4	-	-	-	-	-
Pasture-derived C; 10-20 cm	Monoexponential	F(t) = 4.39 + (24.41-4.39)*exp(t*- (0.030))	0.85	-	-	82.2	NS (0.08)	NS	-	-	-
Pasture-derived C; 10-20 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
Pasture-derived C; 10-20 cm	Single exponential decay	F(t) = 24.1 * exp(t*- (0.02))	0.84	-	-	80.7	***	***	-	-	-
Bulk soil; 20-30 cm	Linear	F(t) = 19.03 - 0.16*t	0.67	***	***	74.5	-	-	-	-	-
Bulk soil; 20-30 cm	Segmented	F(t) = 19.90 - 0.22*t......	0.69	-	**	75.1	-	-	-	***	NS
Bulk soil; 20-30 cm	Monoexponential	F(t) = 3.96 + (19.56-3.96)*exp(t*- (0.021))	0.71	-	-	75.7	NS	NS	-	-	-
Bulk soil; 20-30 cm	Biexponential	No Convergence	-	-	-	-	-	-	-	-	-
OP-derived C; 20-30 cm	Linear	F(t) = -0.20 + 0.07*t	0.68	***	***	45.6	-	-	-	-	-
Pasture-derived C; 20-30 cm	Linear	F(t) = 19.23 - 0.22*t	0.78	***	***	76.9	-	-	-	-	-
Pasture-derived C; 20-30 cm	Monoexponential	F(t) = -41.47 + (19.42+41.47)*exp(t*- (0.048))	0.80	-	-	78.9	NS	NS	-	-	-
Pasture-derived C; 20-30 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
Pasture-derived C; 20-30 cm	Single exponential decay	F(t) = 19.8 * exp(t*- (0.017))	0.79	-	-	77.7	***	***	-		
Bulk soil; 30-50 cm	Linear	F(t) = 29.42 - 0.25*t	0.69	***	***	87.7	-	-	-	-	-
Bulk soil; 30-50 cm	Segmented	F(t) = 27.7 - 0.04*t......	0.70	-	NS	89.1	-	-	-	NS	***
Bulk soil; 30-50 cm	Monoexponential	No convergence	-	-	-	-	-	-	-	-	-
Bulk soil; 30-50 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
OP-derived C; 30-50 cm	Linear	F(t) = 0.02 + 0.10*t	0.57	***	***	66.4	-	-	-	-	-
Pasture-derived C; 30-50 cm	Linear	F(t) = 29 - 0.35*t	0.73	***	***	95.9	-	-	-		
Pasture-derived C; 30-50 cm	Monoexponential	No convergence	-	-	-	-	-	-	-	-	-
Pasture-derived C; 30-50 cm	Biexponential	No convergence	-	-	-	-	-	-	-	-	-
Pasture-derived C; 30-50 cm	Single exponential decay	F(t) = 29.75 * exp(t*- (0.017))	0.69	-	-	99.0	***	***			

[a] e= in Monoexponential function SOC stocks at equilibrium; x1= in Biexponential function SOC stocks of labile pool; x2= in Biexponential function SOC stocks of stable pool; A = in Exponential rise to equilibrium function is OP annual input; c = in single exponential decay function is the initial SOC stocks of pastures.

Table S2. Soil chemical physical and isotopic properties.

	BD	Ca	K	Mg	Na	P	Sum cations	EA	ECEC	BS	pH	13C	15N	CN
Depth (cm)	Mg m^{-3}	mg kg^{-1}					meq 100g^{-1}			%		‰		
Pasture														
0-10	1.27 (±0.06)	21.83 (±14.12)	50.73 (±20.56)	10.44 (±5.27)	9.35 (±4.80)	1.94 (±1.36)	0.37 (±0.18)	3.24 (±0.31)	3.60 (±0.23)	10.20 (±4.78)	4.49 (±0.32)	-17.2 (±1.8)	5.3 (±0.8)	12.3 (±0.6)
10-20	1.34	10.74 (±3.79)	22.49 (±4.34)	4.76 (±0.76)	6.64 (±1.32)	1.90 (±1.86)	0.18 (±0.03)	3.37 (±0.06)	3.55 (±0.10)	5.04 (±0.75)	4.42 (±0.12)	-15.8 (±0.7)	7.3 (±0.6)	12.8 (±0.5)
20-30	1.32	3.07 (±3.08)	15.41 (±2.11)	2.60 (±0.25)	4.99 (±1.45)	1.51 (±0.61)	0.10 (±0.02)	3.17 (±0.31)	3.26 (±0.31)	3.05 (±0.83)	4.59 (±0.14)	-14.3 (±0.4)	8.7 (±0.6)	13.7 (±0.5)
30-50	1.30	1.68 (±1.94)	12.89 (±2.47)	1.81 (±0.22)	3.61 (±2.59)	2.93 (±1.24)	0.07 (±0.01)	2.83 (±0.40)	2.88 (±0.41)	2.51 (±0.09)	4.47 (±0.20)	-13.7 (±0.7)	9.3 (±1.1)	13.3 (±0.5)
OP-12 years														
0-10	1.25	29.89	54.90	26.28	4.60	9.24	0.53	3.86	4.39	12.06	4.04	-18.8	6.1	12.4
10-20.	1.24	5.25	34.02	10.20	6.05	2.76	0.22	3.82	4.04	5.56	4.10	-15.8	7.6	13.2
20-30	1.33	9.65	25.89	6.68	5.88	3.89	0.20	3.59	3.78	5.18	4.20	-15.4	8.6	13.5
30-50	1.40	6.97	21.11	4.22	6.47	10.07	0.15	3.43	3.58	4.25	4.22	-16.3	9.3	12.1
OP-18 years														
0-10	1.24 (±0.04)	87.12 (±48.09)	126.50 (±63.71)	20.21 (±7.75)	4.53 (±3.28)	29.98 (±15.48)	0.95 (±0.39)	3.23 (±0.59)	4.18 (±0.20)	23.00 (±10.79)	4.31 (±0.19)	-19.5 (±0.7)	5.9 (±0.6)	13.6 (±0.3)
10-20.	1.24	34.21 (±24.74)	79.15 (±28.52)	9.48 (±2.27)	4.94 (±2.77)	9.25(±7.15)	0.47 (±0.20)	3.26 (±0.47)	3.73 (±0.31)	12.95 (±6.30)	4.28 (±0.05)	-15.8 (±0.7)	7.7 (±0.4)	15.3 (±0.1)
20-30	1.33	20.89 (±15.47)	48.27 (±13.33)	7.64 (±2.01)	5.09 (±3.44)	6.58 (±2.90)	0.31 (±0.10)	3.16 (±0.64)	3.48 (±0.57)	9.40 (±4.45)	4.41 (±0.15)	-14.3 (±0.3)	9.2 (±0.3)	15.9 (±0.0)
30-50	1.40	11.86 (±0.90)	25.43 (±11.25)	6.44 (±2.61)	3.33 (±3.07)	13.37(±2.58)	0.19 (±0.02)	2.81 (±0.35)	3.00 (±0.35)	6.43 (±0.93)	4.48 (±0.13)	-13.6 (±0.2)	10.4 (±0.3)	15.9 (±0.3)
OP-30 years														
0-10	1.45 (±0.11)	93.77 (±16.83)	26.78 (±3.08)	15.76 (±11.78)	2.71 (±2.79)	14.96 (±1.03)	0.68 (±0.18)	2.22 (±0.12)	2.90 (±0.06)	23.40 (±5.65)	4.24 (±0.11)	-23.2 (±0.8)	5.6 (±0.4)	12.0 (±0.1)
10-20.	1.38	29.82 (±14.55)	15.93 (±1.74)	4.13 (±2.08)	3.03 (±4.28)	3.76 (±0.92)	0.24 (±0.07)	2.48 (±0.15)	2.72 (±0.21)	8.64 (±1.74)	4.45 (±0.01)	-18.8 (±0.3)	7.3 (±0.5)	13.1 (±0.3)
20-30	1.47	14.84 (±11.08)	16.40 (±2.19)	3.23 (±2.36)	10.31 (±8.89)	3.85 (±1.17)	0.17 (±0.14)	2.62 (±0.09)	2.79 (±0.23)	5.98 (±4.56)	4.51 (±0.02)	-16.2 (±0.4)	8.6 (±0.4)	13.4 (±0.3)
30-50	1.51	2.25 (±3.19)	11.44 (±1.99)	1.39 (±0.83)	1.43 (±2.09)	3.77 (±2.48)	0.06 (±0.01)	2.63 (±0.06)	2.69 (±0.07)	2.17 (±0.27)	4.45 (±0.04)	-15.6 (±0.4)	9.1 (±0.2)	12.5 (±0.7)
OP-32 years														
0-10	1.43	93.25	79.96	36.00	2.78	20.66	0.98	2.06	3.05	32.25	4.10	-22.80	6.2	12.1
10-20.	1.22	27.38	49.20	9.84	3.49	7.04	0.36	2.37	2.73	13.17	4.19	-18.50	7.5	12.8
20-30	1.34	13.84	42.13	5.78	3.69	9.28	0.24	2.56	2.80	8.60	4.18	-16.60	8.6	13.4
30-50	1.50	4.93	35.58	5.03	3.93	5.29	0.17	2.56	2.74	6.39	4.18	-15.70	9.1	12.1
OP-45 years														
0-10	1.42 (±0.05)	152.55 (±96.30)	90.18 (±21.98)	30.49 (±8.49)	3.83 (±0.68)	16.43 (±3.49)	1.26 (±0.60)	2.25 (±0.21)	3.52 (±0.44)	35.00 (±11.96)	4.30 (±0.17)	-23.5 (±0.2)	6.2 (±0.2)	10.2 (±0.6)
10-20.	1.45	84.39 (±43.08)	57.59 (±11.59)	13.20 (±3.61)	2.72 (±2.31)	3.50 (±0.47)	0.69 (±0.26)	2.56 (±0.38)	3.25 (±0.64)	20.80 (±3.55)	4.33 (±0.17)	-18.4 (±0.4)	7.2 (±0.2)	10.0 (±0.5)
20-30	1.40	62.90 (±25.02)	59.85 (±6.27)	11.19 (±3.14)	3.86 (±3.18)	1.91 (±0.83)	0.58 (±0.15)	2.86 (±0.35)	3.43 (±0.35)	16.89 (±4.37)	4.33 (±0.15)	-15.8 (±0.4)	8.1 (±0.2)	10.5 (±1.0)
30-50	1.50	34.56 (±19.82)	50.72 (±12.12)	7.83 (±3.10)	4.48 (±3.80)	0.70 (±0.64)	0.39 (±0.14)	2.77 (±0.38)	3.16 (±0.31)	12.45 (±5.11)	4.46 (±0.27)	-15.1 (±0.3)	8.2 (±0.2)	9.7 (±0.3)
OP-56 years														
0-10	1.15 (±0.08)	151.57 (±39.77)	110.77 (±29.88)	59.41 (±23.27)	9.19 (±1.32)	14.35 (±8.07)	1.58 (±0.43)	3.66 (±0.27)	5.24 (±0.64)	29.73 (±4.73)	4.40 (±0.16)	-24.3 (±0.3)	6.5 (±0.3)	9.9 (±0.3)
10-20.	1.17	61.31(±10.22)	66.82 (±17.89)	23.61 (±8.24)	5.67 (±3.02)	1.26 (±0.77)	0.70 (±0.15)	4.39 (±0.20)	5.09 (±0.32)	13.65 (±2.42)	4.34 (±0.15)	-20.4 (±0.6)	7.1 (±0.1)	9.9 (±1.4)
20-30	1.32	37.18 (±8.00)	49.46 (±8.26)	17.47(±5.26)	6.74 (±4.59)	0.25 (±0.25)	0.49 (±0.09)	4.20 (±0.50)	4.69 (±0.57)	10.35 (±1.08)	4.42 (±0.08)	-19.5 (±0.4)	7.1 (±0.2)	8.6 (±2.0)
30-50	1.39	26.74 (±10.32)	36.51 (±10.03)	16.18 (±6.51)	6.24 (±4.38)	0.25 (±0.17)	0.39 (±0.11)	3.57 (±0.66)	3.95 (±0.73)	9.85 (±1.73)	4.54 (±0.16)	-20.8 (±0.2)	6.6 (±0.2)	5.9 (±0.3)

EA: exchangeable acidity; ECEC: effective cation exchange capacity; BS; base saturation; pH: in water (1:2.5).

Mean values (n=3 for all sites, except for 30 years oil palm (OP-30 years) where n=2, standard deviation between brackets; where no SD appears, values correspond to a single measurement

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Figure S1. Schematic representation of oil palm management zones in a mature plantation (9 years old). + symbols refer to the magnitude of the input, *i.e.* fertilization +++, is high fertilizers application and fertilization +, is low fertilizers application.

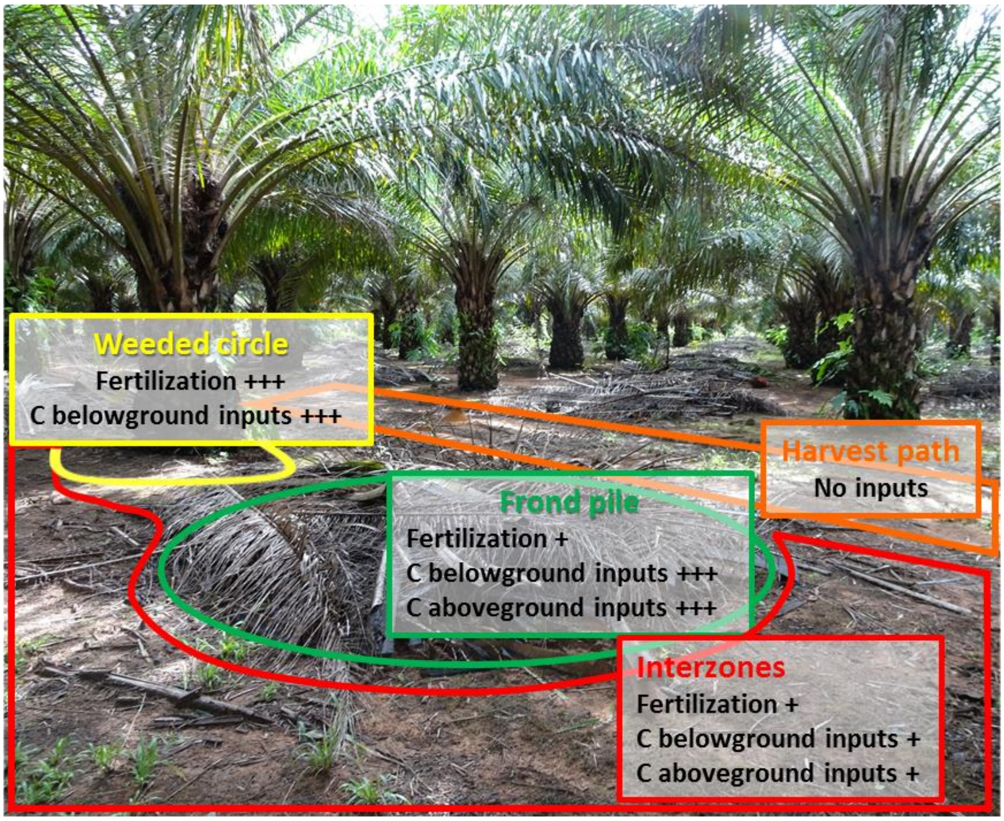
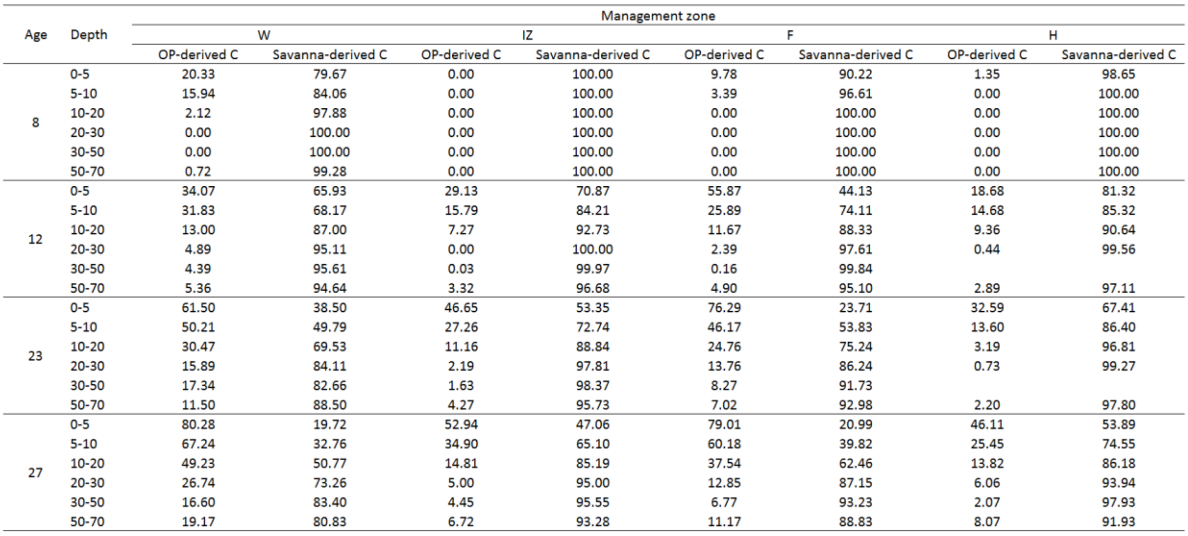


Table S1. Rates of change in bulk SOC stocks (Mg ha^{-1} yr^{-1}) and SE across one OP-rotation cycle (27-years) by layer. Results of ANCOVA using linear mixed-models. *P*-values are indicated with the respective symbols *** for *P*<0.001, ** for *P*<0.01, * for *P*<0.05, NS for *P*>0.05. NA stays for not applicable. When the interaction between time after conversion and management type was significant the model with the effect of time after savanna conversion within management type was used.

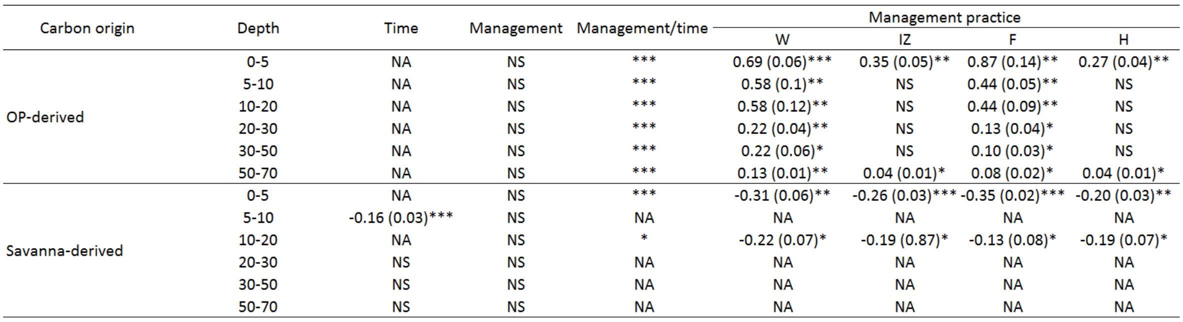
Depth	Time	Management	Management/time	Management practice			
				W	IZ	F	H
0-5	NA	NS	***	0.37 (0.11)*	NS	0.53 (0.14)*	NS
5-10	NA	NS	***	0.40 (0.13)*	NS	0.25 (0.03)*	NS
10-20	NA	NS	***	0.36 (0.08)*	NA	0.30 (0.06)**	NA
20-30	NS	NS	NA	NA	NA	NA	NA
30-50	NS	NS	NA	NA	NA	NA	NA
50-70	NS	NS	NA	NA	NA	NA	NA

Table S2. Proportion (%) of OP and savanna-derived C by layer and time after savanna conversion at each management zone. When the interaction between time after conversion and management type was significant, the model with the effect of time after savanna conversion within management type was used.



Age	Depth	Management zone							
		W		IZ		F		H	
		OP-derived C	Savanna-derived C	OP-derived C	Savanna-derived C	OP-derived C	Savanna-derived C	OP-derived C	Savanna-derived C
8	0-5	20.33	79.67	0.00	100.00	9.78	90.22	1.35	98.65
	5-10	15.94	84.06	0.00	100.00	3.39	96.61	0.00	100.00
	10-20	2.12	97.88	0.00	100.00	0.00	100.00	0.00	100.00
	20-30	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
	30-50	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00
	50-70	0.72	99.28	0.00	100.00	0.00	100.00	0.00	100.00
12	0-5	34.07	65.93	29.13	70.87	55.87	44.13	18.68	81.32
	5-10	31.83	68.17	15.79	84.21	25.89	74.11	14.68	85.32
	10-20	13.00	87.00	7.27	92.73	11.67	88.33	9.36	90.64
	20-30	4.89	95.11	0.00	100.00	2.39	97.61	0.44	99.56
	30-50	4.39	95.61	0.03	99.97	0.16	99.84		
	50-70	5.36	94.64	3.32	96.68	4.90	95.10	2.89	97.11
23	0-5	61.50	38.50	46.65	53.35	76.29	23.71	32.59	67.41
	5-10	50.21	49.79	27.26	72.74	46.17	53.83	13.60	86.40
	10-20	30.47	69.53	11.16	88.84	24.76	75.24	3.19	96.81
	20-30	15.89	84.11	2.19	97.81	13.76	86.24	0.73	99.27
	30-50	17.34	82.66	1.63	98.37	8.27	91.73		
	50-70	11.50	88.50	4.27	95.73	7.02	92.98	2.20	97.80
27	0-5	80.28	19.72	52.94	47.06	79.01	20.99	46.11	53.89
	5-10	67.24	32.76	34.90	65.10	60.18	39.82	25.45	74.55
	10-20	49.23	50.77	14.81	85.19	37.54	62.46	13.82	86.18
	20-30	26.74	73.26	5.00	95.00	12.85	87.15	6.06	93.94
	30-50	16.60	83.40	4.45	95.55	6.77	93.23	2.07	97.93
	50-70	19.17	80.83	6.72	93.28	11.17	88.83	8.07	91.93

Table S3. Rates of change of OP- and savanna-derived C (Mg ha^{-1} yr^{-1}) and SE across one OP-rotation cycle (27-years) by layer. Results of ANCOCA linear mixed-models. *P*-values are indicated with the respective symbols *** for *P*<0.001, ** for *P*<0.01, * for *P*<0.05, NS for *P*>0.05. NA stays for not applicable. When the interaction between time after conversion and management type was significant, the model with the effect of time after savanna conversion within management type was used.



Carbon origin	Depth	Time	Management	Management/time	Management practice			
					W	IZ	F	H
OP-derived	0-5	NA	NS	***	0.69 (0.06)***	0.35 (0.05)**	0.87 (0.14)**	0.27 (0.04)**
	5-10	NA	NS	***	0.58 (0.1)**	NS	0.44 (0.05)**	NS
	10-20	NA	NS	***	0.58 (0.12)**	NS	0.44 (0.09)**	NS
	20-30	NA	NS	***	0.22 (0.04)**	NS	0.13 (0.04)*	NS
	30-50	NA	NS	***	0.22 (0.06)*	NS	0.10 (0.03)*	NS
	50-70	NA	NS	***	0.13 (0.01)**	0.04 (0.01)*	0.08 (0.02)*	0.04 (0.01)*
Savanna-derived	0-5	NA	NS	***	-0.31 (0.06)**	-0.26 (0.03)***	-0.35 (0.02)***	-0.20 (0.03)**
	5-10	-0.16 (0.03)***	NS	NA	NA	NA	NA	NA
	10-20	NA	NS	*	-0.22 (0.07)*	-0.19 (0.87)*	-0.13 (0.08)*	-0.19 (0.07)*
	20-30	NS	NS	NA	NA	NA	NA	NA
	30-50	NS	NS	NA	NA	NA	NA	NA
	50-70	NS	NS	NA	NA	NA	NA	NA

When values are 0 or 100, they were put as such due to slightly negative or beyond 100 values.

Table S4. Rates of change in soil chemical properties and SE within given soil layers over one OP rotation cycle (27-years). Results of ANCOCA linear mixed-models. *P*-values are indicated with the respective symbols *** for *P*<0.001, ** for *P*<0.01, * for *P*<0.05, NS for *P*>0.05. NA stays for not applicable. When the interaction between time after conversion and management type was significant, the model with the effect of time after savanna conversion within management type was used.

Soil properties	Depth	Time	Management	Management/time	Management practice			
					W	IZ	F	H
Sum cations	0-5	NA	NS	***	0.22 (0.05)**	NS	0.25 (0.03)**	NS
	5-10	NA	NS	***	0.11 (0.03)*	NS	0.10 (0.02)*	NS
	10-20	0.03 (0.008) ***	NS	NA	NA	NA	NA	NA
	20-30	0.02 (0.006)**	NS	NA	NA	NA	NA	NA
	30-50	NS	NS	NA	NA	NA	NA	NA
	50-70	0.01 (0.005)*	NS	NA	NA	NA	NA	NA
Bray-P	0-5	NA	NS	***	7.36 (1.85)*	NS	NS	NS
	5-10	NA	NS	***	3.59 (0.11)***	NS	0.25 (0.14)*	NS
	10-20	NA	NS	***	1.28 (0.42)*	NS	NS	NS
	20-30	NA	NS	***	0.47 (0.16)*	NS	NS	NS
	30-50	NS	NS	NA	NA	NA	NA	NA
	50-70	NS	NS	NA	NA	NA	NA	NA
pH	0-5	NS	NS	NA	NA	NA	NA	NA
	5-10	NS	NS	NA	NA	NA	NA	NA
	10-20	NS	NS	NA	NA	NA	NA	NA
	20-30	NS	NS	NA	NA	NA	NA	NA
	30-50	NA	NS	*	-0.04(0.02)*	NS	NS	NS
	50-70	NS	NS	NA	NA	NA	NA	NA
C content	0-5	NA	NS	***	0.08 (0.02)***	NS	0.13 (0.03)**	NS
	5-10	NA	NS	***	0.08 (0.01)***	NS	0.05 (0.008)*	NS
	10-20	NA	NS	***	0.04 (0.007)**	NS	0.04 (0.005)**	NS
	20-30	NA	NS	***	0.01 (0.007)*	NS	NS	NS
	30-50	NS	NS	NA	NA	NA	NA	NA
	50-70	NA	NS	**	0.13 (0.003)*	NS	0.015 (0.005)*	NS
13C	0-5	NA	NS	***	-0.40 (0.03)***	-0.30 (0.05)***	-0.45 (0.07)***	-0.23 (0.04)***
	5-10	NA	NS	***	-0.40 (0.03)***	-0.19 (0.04)***	-0.33 (0.04)**	-0.13 (0.04)*
	10-20	NA	NS	***	-0.26 (0.04)**	NS	-0.20 (0.04)**	NS
	20-30	NA	NS	***	-0.14 (0.03)*	NS	-0.09 (0.03)*	NS
	30-50	NA	NS	***	-0.11 (0.02)**	NS	-0.05 (0.02)*	NS
	50-70	NA	NS	***	-0.11 (0.02)**	-0.04 (0.01)*	-0.06 (0.0)**	-0.04 (0.02)*
15N	0-5	NS	**	NA	NA	NA	NA	NA
	5-10	NS	*	NA	NA	NA	NA	NA
	10-20	NS	NS	NA	NA	NA	NA	NA
	20-30	NS	NS	NA	NA	NA	NA	NA
	30-50	NS	NS	NA	NA	NA	NA	NA
	50-70	NS	NS	NA	NA	NA	NA	NA

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Figure S1: Locations of research sites.

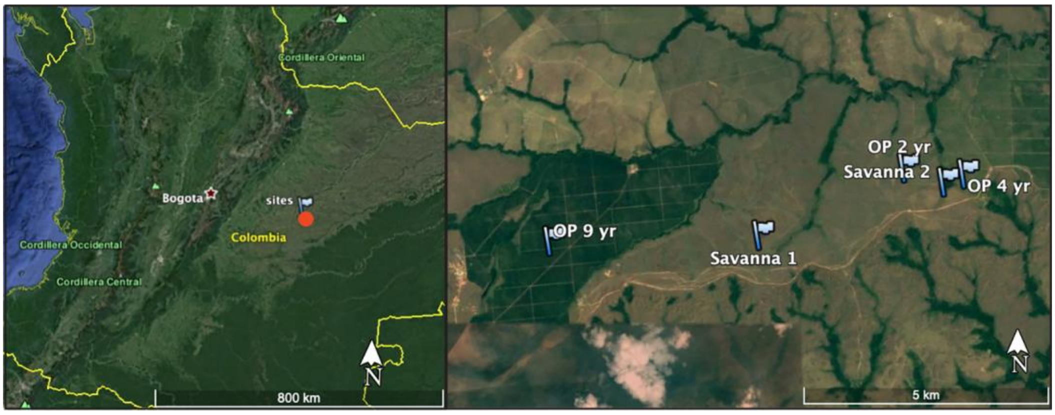

Figure S2: Development of oil palm roots depending on distance from the palm, soil depth and plantation age. Percentage of sampling points with the presence of oil palm coarse or fine roots in the 2- and 4-years old plantations.

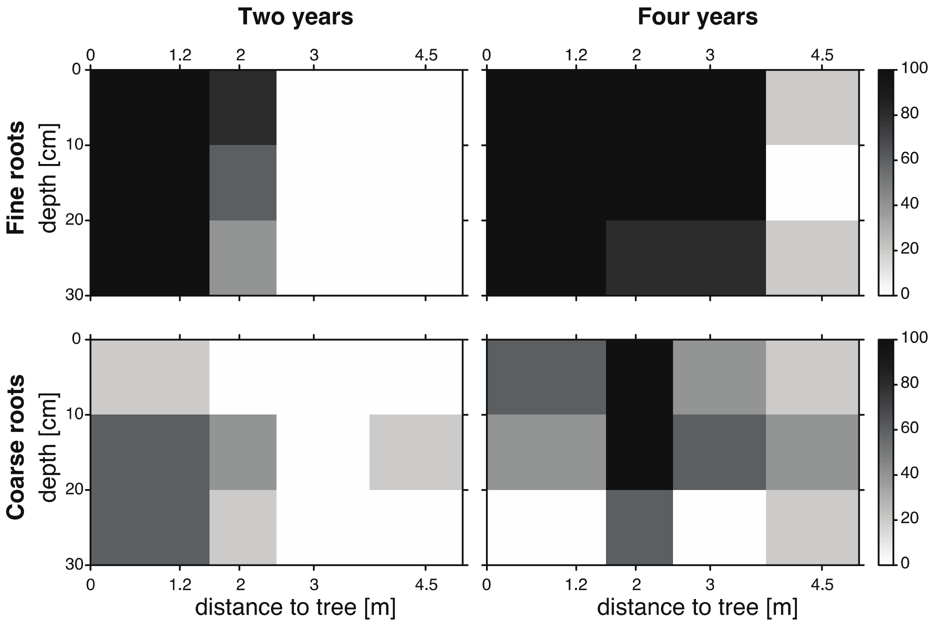

Figure S3: Full model tested with path analysis on the top 10 cm depth of the three management zones located at 4.5 m away from palms (HP, IZ and FP) in the 9-years old plantation. Phosphorous availability was used as proxy of nutrient availability. Soil C:N ratio was used as proxy for soil organic matter (SOM) quality. Scores of samples of the first principal component of a principle component analysis with all microbial related variables were used to characterized soil microorganisms (Supplementary Figure 3).

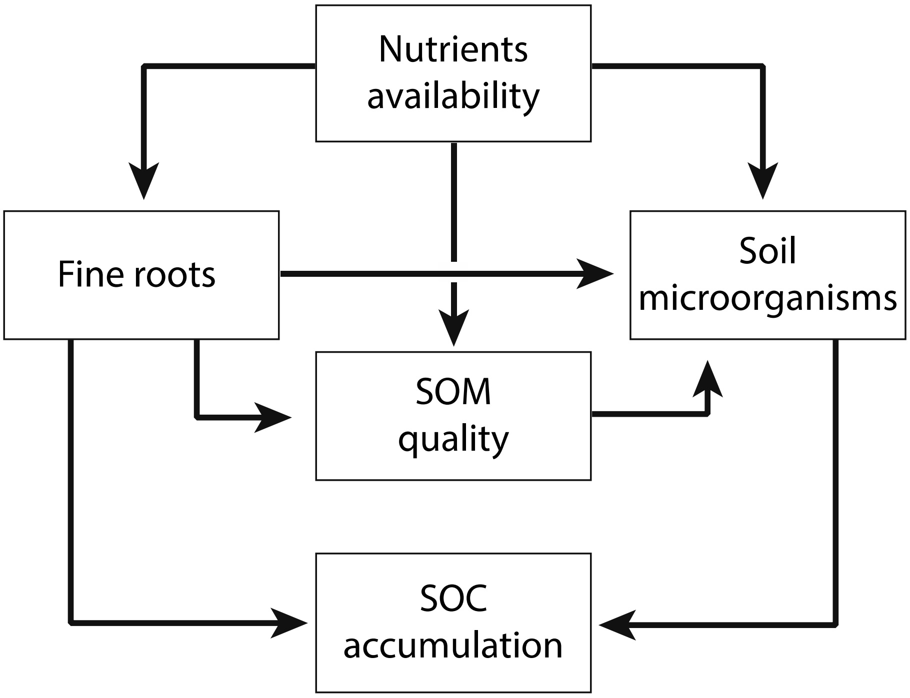

Figure S4: Principal component analysis on microbially-related parameters: microbial biomass C (Cmic), microbial N (Nmic), C:N ratio (CNmic), basal respiration (BR), specific respiration (specific_resp = BR/SOC content) and metabolic quotient (microbialQ = BR/Cmic). Scores of samples on the 1st axis were used as proxies for soil microorganisms in the path analysis.

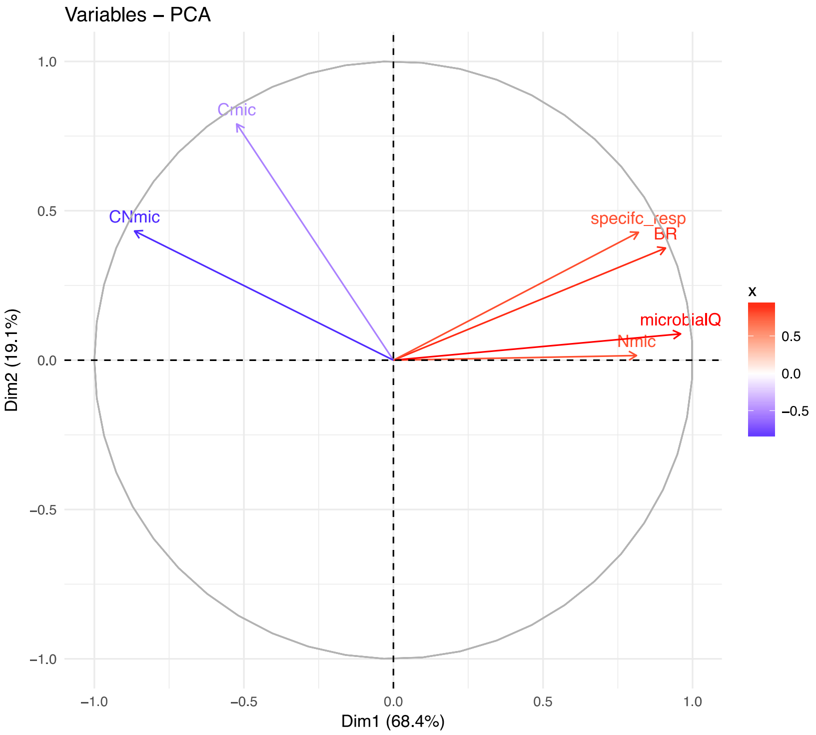

Table S1. Mean ± standard error of soil, roots and microbial variables in the top 10 cm in the harvest path (HP), interzones (IZ), frond piles (FP) and weeded circles (WC) of the 9-years old plantation.

variables (0-10 cm)	**units**	**Management zones**							
		HP		**IZ**		**FP**		**WC**	
SOC stocks	*kg m*$^{-2}$	0.81 ± 0.07	*ab*	0.78 ± 0.06	*a*	1.03 ± 0.06	*c*	0.95 ± 0.02	bc
C3 stocks	*kg m*$^{-2}$	0.28 ± 0.03	*a*	0.21 ± 0.04	*a*	0.47 ± 0.04	*b*	0.46 ± 0.05	b
C4 stocks	*kg m*$^{-2}$	0.54 ± 0.05	*a*	0.57 ± 0.02	*a*	0.56 ± 0.04	*a*	0.49 ± 0.04	a
C:N ratio		13.1 ± 0.1	*a*	13.4 ± 0.1	*a*	14.4 ± 0.3	*b*	13.0 ± 0.3	a
DOC	*mg kg*$^{-1}$	55 ± 2	*ab*	65 ± 7	*b*	65 ± 3	*b*	43 ± 5	a
Available P	*mg kg*$^{-1}$	11.1 ± 2.6	*a*	15.9 ± 3.0	*ab*	19.7 ± 3.0	*b*	151.1 ± 27.1	c
Fine root biomass	*kg m*$^{-2}$	0.41 ± 0.04	*a*	0.40 ± 0.05	*a*	0.81 ± 0.18	*b*	0.63 ± 0.06	b
Net C3 stabilization	*% cumulative fine roots*	27 ± 3	*b*	22 ± 4	*ab*	23 ± 2	*ab*	16 ± 2	a
Microbial biomass C	*mg kg*$^{-1}$	99 ±10	*a*	136 ± 9	*b*	101 ± 5	*a*	82 ± 7	a
Microbial biomass N	*mg kg*$^{-1}$	5.3 ± 0.7	*a*	5.1 ± 0.5	*a*	10.6 ± 1.0	*b*	5.0 ± 0.6	a
Microbial biomass C:N		19.4 ± 2.3	*b*	27.3 ± 3.4	*c*	9.8 ± 0.8	*a*	16.6 ± 1.4	ab
Basal respiration	*mg C-CO*$_2$ *kg*$^{-1}$ *soil d*$^{-1}$	1.1 ± 0.3	*a*	1.0 ± 0.1	*a*	1.7 ± 0.2	*b*	1.5 ± 0.2	ab
Specific respiration	*mg C-CO*$_2$ *g*$^{-1}$ *C-SOC d*$^{-1}$	0.17 ± 0.04	*a*	0.16 ± 0.02	*a*	0.21 ± 0.03	*a*	0.20 ± 0.2	a
Metabolic quotient	*mg C-CO*$_2$ *mg*$^{-1}$ *C-Cmic d*$^{-1}$	11.0 ± 2.3	a	7.4 ± 1.0	b	17.0 ± 2.0	b	19.2 ± 3.9	a

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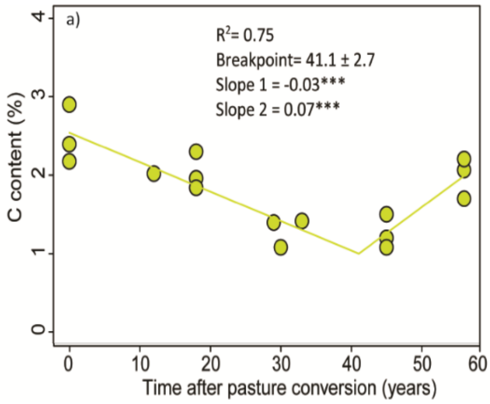
Table S1. List of linear and non-linear regressions. Parameters describe C dynamics in each of the five isolated fraction. Models in bold letters are the ones that best described the dynamics of C in each fraction.

Parameter	Soil fraction	Model type	Function	R2	P value of age	Shapiro test	AIC	k (yr^{-1})	C_0	A	a	b
C concentration	DOC	Linear	F(t) = 0.32 - 0.002*t	0.22	0.0613	0.9065	NA	NA	NA	NA	NA	NA
	POM	**Linear**	**F(t) = 0.58 + 0.02*t**	**0.39**	**0.0128 ***	**0.65**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	rSOC	Linear	F(t) = 2.86 + 0.023*t	0.13	NS	NA	NA	NA	NA	NA	NA	NA
	SC	**Linear**	**F(t) = 4.41 + 0.09*t**	**0.32**	**0.0395 ***	**0.4803**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	SA	Linear	F(t) = 11.89 - 0.23*t	0.55	0.005530 **	0.05004	61.63	NA	NA	NA	NA	NA
	SA	**Single exponential decay**	**F(t) = 67.4 * exp(t*- 0.12)**	**0.91**	**NA**	**NA**	**45.42**	***	*	**NA**	**NA**	**NA**
	SA	$y = a+e^{-kt}$	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SA	$y = e^{-kt}$	F(t) = exp(t*- (-0.015))	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SA	Rise to maximun	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Relative proportion of total SOC	DOC	Linear	NS	NA	NA	NA	NA	NA	NA	NA	NA	NA
	POM	Linear	F(t) = 3.97 + 0.13*t	0.45	0.007**	0.006485	61.64	NA	NA	NA	NA	NA
	POM	**Rise to equilibium**	**F(t) = -0.51 * exp (-0.042 * t) + 0.51)/0.042**	**0.61**	**NA**	**NA**	**58.14**	*	**NA**	**	**NA**	**NA**
	POM	**Rise to maximun**	**F(t) = 12.03 * (1 - exp (-0.04 * t))**	**0.61**	**NA**	**NA**	**58.14**	**NA**	**NA**	**NA**	***	*
	rSOC	Linear	F(t) = 17.39 + 0.16*t	0.28	0.05450 .	0.34	65.21	NA	NA	NA	NA	NA
	rSOC	**Rise to equilibium**	**F(t) = 2.20 * exp (-0.085 * t) + 2.20)/0.085**	**0.51**	**NA**	**NA**	**62.17**	**	**NA**	**	**NA**	**NA**
	rSOC	**Rise to maximun**	**F(t) = 25.93 * (1 - exp (-0.085 * t))**	**0.51**	**NA**	**NA**	**62.17**	**NA**	**NA**	**NA**	***	**
	SC	Linear	F(t) = 27.95 + 0.53*t	0.47	0.01203 *	0.338	83.3	NA	NA	NA	NA	NA
	SC	**Rise to equilibium**	**F(t) = 3.30 * exp (-0.06 * t) + 3.30)/0.06**	**0.72**	**NA**	**NA**	**77.63**	**	**NA**	***	**NA**	**NA**
	SC	**Rise to maximun**	**F(t) = 58.21 * (1 - exp (-0.06 * t))**	**0.72**	**NA**	**NA**	**77.63**	**NA**	**NA**	**NA**	***	**
	SA	Linear	F(t) = 63.52 - 1.19*t	0.59	0.003535 **	0.2273	96.26	NA	NA	NA	NA	NA
	SA	**Single exponential decay**	**F(t) = 243.12 * exp(t*- (0.10))**	**0.85**	**NA**	**NA**	**86.59**	**	*	**NA**	**NA**	**NA**
	SA	$y = a+e^{-kt}$	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SA	$y = e^{-kt}$	F(t) = exp(t*- (-0.04))	NA	NA	NA	NA	NA	NA	NA	NA	NA
	SA	Rise to maximun	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Fraction mass	**POM**	**Linear**	**F(t) = 0.051 + 0.002*t**	**0.39**	**0.0137 ***	**0.49**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	SC	**Linear**	**F(t) = 2.95 + 0.37*t**	**0.85**	**4.09e-06 *****	**0.49**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	SA	**Linear**	**F(t) = 26.05 - 0.36*t**	**0.87**	**2.20e-06 *****	**0.36**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
C3 concentration	DOC	Linear	F(t) = 0.012 + 0.003*t	0.29	NS	NA	NA	NA	NA	NA	NA	NA
	POM	**Linear**	**F(t) = 0.27 + 0.017*t**	**0.50**	**0.00435 ****	**0.53**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	rSOC	**Linear**	**F(t) = -0.84 + 0.06*t**	**0.94**	**4.38e-07 *****	**0.17**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	SC	**Linear**	**F(t) = -1.26 + 0.12*t**	**0.83**	**5.56e-05 *****	**0.53**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
	SA	**Linear**	**F(t) = 1.50 - 0.020*t**	**0.40**	**0.04005 ***	**0.88**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
C4 concentration	DOC	NA	F(t) = -0.09 + 0.0001*t	-0.50	NS	NA	NA	NA	NA	NA	NA	NA
	POM	Linear	F(t) = 0.31 + 0.005*t	0.11	NS	NA	NA	NA	NA	NA	NA	NA
	rSOC	Linear	F(t) = 3.71 - 0.027*t	0.13	NS	NA	NA	NA	NA	NA	NA	NA
	SC	Linear	F(t) = 5.68 - 0.023*t	-0.030	NS	NA	NA	NA	NA	NA	NA	NA
	SA	Linear	F(t) = 10.49 - 0.21*t	0.480	0.01070 *	0.0565	NA	NA	NA	NA	NA	NA
	SA	**Single exponential decay**	**F(t) = 96.81 * exp(t*- 0.15)**	**0.91**	**NA**	**NA**	**44.46**	***	*	**NA**	**NA**	**NA**

Figure S1. Bulk soil C content dynamics over two OP rotation cycles following pasture conversion in the topsoil (0-10 cm). Taken from Quezada et al., 2019.



9 Appendix

“If you want 1 year of ***prosperity****, grow grain. If you want 10 years of prosperity, grow trees. If you want* ***100 years*** *of prosperity,* ***grow people****.”*

Chinese Proverb

9.1 Appendix 1: Screening of Potential Nitrifiers Activity in Cultivated Oil Palm Plantation Soils

Juan Carlos Quezada[a] and Ashly Arevalo[b]

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[b] Tropical Frages Group, CIAT, Cali, Colombia

Here I present the results concerning a soil incubation experiment carried out to determine changes over time in soils nitrifiers activity following land use change from savanna or pasture to oil palm plantations in the well-drained savanna area (Altillanura) of Los Llanos in Colombia. This work was done in cooperation with the group of the Tropical Forages Program at the CIAT headquarters facilities in Cali, Colombia.

9.1.1 Materials and Methods

Table 1, presents the complete description of the samples selected for this study. Soil samples were chosen to represent a range of conditions, which could allow us to examine the effects of land-use change on the stability of residual biological nitrification inhibition (BNI) effects in oil palm (OP) plantations. In this sense, soil samples of OP plantations of 2, 4 and 8 years old from commercial plantations located in the well-drained savanna region of Los Llanos (Altillanura) were considered. Samples from reference sites (two Brachiaria pasture types and one semi-natural savanna area) located in the vicinity and adjacent to the OP plantations were included as well. Composite soil samples were taken from the surface layer (0 – 0.05 m depth) of all sites using a 6-cm-diam. auger. In the three reference sites ten cores were taken along a N-S transect containing representative vegetation cover of each study site. At each OP study site ten oil palm trees within a representative area were sampled at points randomly selected within a design stratified by management zones (for detail description of management zones see the research proposal document).

For the 2 and 4 years old plantations derived from savanna areas two management zones were included: the between palm area (S) and the weeded circle (W) zone. For the mature plantations (8 yrs. old) the S zone was replaced by the frond pile zone (F). This was done due to the lack of cover crops presence in OP plantations of this age. Moreover, the F zones presents an opportunity to evaluate the influence of biomass inputs derived from the pruning practice on nitrification rates. OP plantations in formerly Brachiaria pasture areas plantations of 4 and 8 years old were considered. For the former, samples from the W and S zones were included, whereas samples from the W, F and H zones were considered for the mature planation. At this latter site, there was still presence of Brachiaria pasture (possibly *Brachiaria decumbes*) in the H zone at the moment of sampling. Unfortunately, OP plantations of 2 years old established in previously Brachiaria pasture areas were not found, therefore they were not included in this study.

For comparison purposes a sample from a BNI experimental site managed by CIAT was included (Maiz-CYMMIT). In this experiment a corn crop, negative control, with high nitrification rates was sown following a Brachiaria pasture phase of about 15 years. In order to evaluate nitrification rates in the above described samples the CIAT protocol: “Protocol for soil incubation experiments to study nitrifiers activity potential in soils” was performed (Annex 1). This soil incubation procedure involves the following conditions:

- Air-dried and sieved soil samples (passed through a 2-mm sieve) were used.
- Soil samples were replicated three times (technical replicates). Mean values are reported in the results section for each measurement time for each sample (159 samples in total).
- The incubation was run for 27 days in dark vials where 5 g of sample were placed. Samples were retrieved at 11, 19 and 27 d of soil incubation. Before the beginning of the incubation the initial concentrations of NO_3-N and NH_4-N were determined (basal measurements). For the following three dates, inorganic N concentrations were determined using KCl extraction and the protocol used at CIAT for determination of this parameter.
- The synthetic inhibitor dicyandiamide (DCD) was added to one sample in order to inhibit nitrification (negative control of nitrification).
- The slope of the linear regressions (x = incubation time and y =evolution of NO_3 or NH_4 concentrations) was considered as the nitrification rate of each sample.

9.1.2 Results and Discussion

As this study was designed with the purpose of getting an overview of the impacts of land-use change (from savanna or Brachiaria pasture areas to OP plantations) on nitrification rates over time no field/sites replicates were considered, rather three technical replicates in the laboratory were performed and their mean values are reported here.The initial NO_3-N concentrations in our reference samples were nearly 8-fold greater than those typically found in soils of this kind, while the NH_4-N concentrations were similar to those reported in the literature (Bradley et al., 1988). Unlike many other agricultural production systems, tropical acidic soils have shown to have prevalence of NH_4-N over NO_3-N (Subbarao et al., 2012; Rao et al., 2016). In our soils, average NH_4-N:NO_3-N ratio did not vary greatly from the expected values (Table 1). A possible explanation for the slightly superior concentration of NO_3-N over NH_4-N is that accumulation of the former could have taken place as samples were not kept under cold storage after sampling. For future research, it will be better to conduct chemical extractions in the field so that any transformation of inorganic N can be prevented.

Table 1. Initial NH_4-N and NO_3-N concentrations in the 15 samples considered for this incubation study.

Land-use	Inorganic N NH_4-N	 NO_3-N	 NH_4-N: NO_3-N
	-----mg kg^{-1} soil-----		Ratio
Savanna 1	4.2	5.0	0.8
Brachiaria 1 (decumbens)	2.5	6.9	0.4
Brachiaria 2(mulato)	3.5	6.0	0.6
OP (2y) W Savanna	5.5	12.5	0.4
OP (2y) S Savanna	5.0	6.0	0.8
OP (4y) W Savanna	13.4	13.7	1.0
OP (4y) S Savanna	7.9	5.2	1.5
OP (8y) W Savanna	5.5	5.7	1.0
OP (8y) F Savanna	8.7	5.7	1.5
OP (8y) W Brachiaria	3.8	5.5	0.7
OP (8y) F Brachiaria	7.4	6.2	1.2
OP (8y) H Brachiaria	5.5	7.4	0.7
OP (4y) W Brachiaria	6.8	7.2	0.9
OP (4y) S Brachiaria	4.6	5.7	0.8
Maiz CIMMYT	4.5	9.3	0.5
Mean	5.9	7.2	0.9
CV (%)	45%	37%	40%
SE	0.69	0.68	0.09

Rates of nitrification for the 15 evaluated soils are reported in Figure 1. The three reference sites showed reduced nitrification rates compared to all the OP plantation soils except for the one from the F management zone of a mature plantation established on formerly savanna land. Also, 6 out of the total 11 OP plantations samples showed lower nitrification rates in comparison to the negative control for BNI (Maiz-CIMMYT sample). Nitrification rate values were similar between the added DCD (synthetic chemical inhibitor) sample, one forage grass type (possibly *Brachiaria decumbes*) and the savanna sample (ranged from 0.11 to 0.17 mg NO_3-N kg^{-1} soil d^{-1}). The nitrification rate of the other Brachiaria type pasture (possibly *Brachiaria mulato*) was about twofold that of the *Brachiaria decumbes*. The low nitrification rates obtained for the three types of vegetation covers of the reference sites of this study are comparable to those previously reported by CIAT and JIRCAS studies (Rondon et al., 2005; Subbarao et al.,2009; Moreta et al., 2014

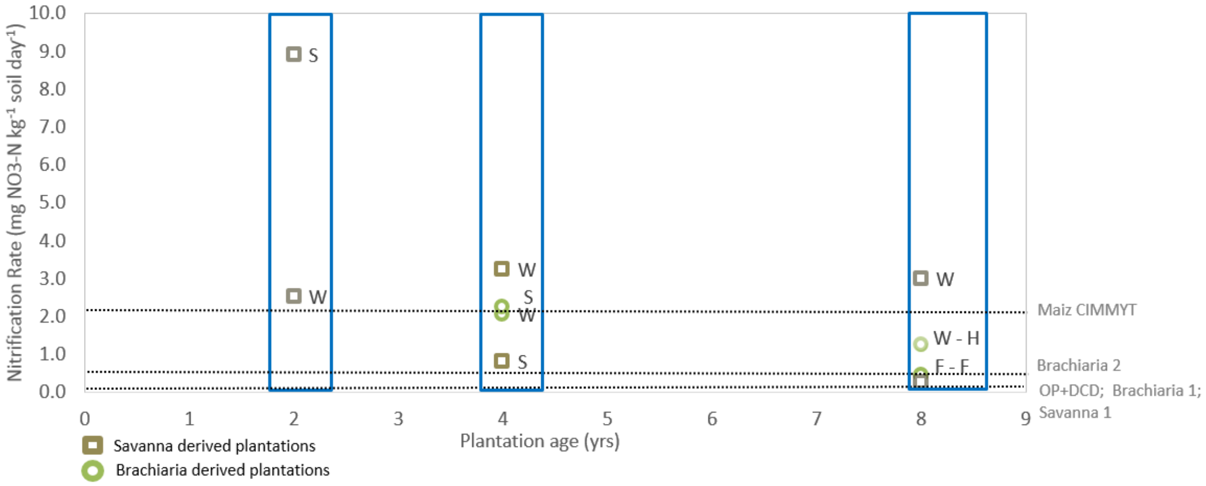


Fig. 1. Nitrification rates of soils from: three reference sites (1 savanna and 2 Brachiaria types dotted horizontal lines), 11 oil palm plantations (with four management zones W, F, S and H) and two control samples (DCD added OP sample and Maiz-CIMMYT dotted horizontal lines) incubated aerobically at 25°C for 27 days.

An apparent range of inhibition of nitrification was observed among samples that contained different leguminous cover crops species (Kudzu (*Pueraria phaseoloides*) and *Desmodium spp.*) at the moment of sampling in the S management zone of the 2 and 4 years old OP plantations converted from savanna. In fact, the sample containing kudzu cover crop presented the highest nitrification rate value among all the analyzed samples. Likewise, samples that had Brachiaria vegetation at the moment of sampling showed variable rates of nitrification (ranged from 1.3 to 2.3 mg NO_3-N kg^{-1} soil d^{-1}). Based on the results of this screening I observed that mean nitrification rate values in OP soils varied depending on the previous vegetation cover. The lowest mean nitrification rate values were found in OP plantations established on both formerly Brachiaria pastures sites and savannas. Also, nitrification rates across management zones and plantation age (4 and 8yrs old) of both the savanna and Brachiaria derived sites were lower compared to the Maiz-CIMMYT sample (Fig. 2).

Our findings together are of interest because the Maiz-CIMMYT soil is considered to have large nitrification rates (Moreta et al., 2014) and the OP soils tested here were converted from either natural savanna or Brachiaria pastures 4 or 8 years ago, (depending on the sample). Most samples were found to have nitrification rates lower than the maize field that was converted about four years ago from a Brachiaria pasture phase. Even more, four samples had as low nitrification rates as the added DCD samples. This could be a clear indicative that the dynamics of the BNI legacy effect may vary depending on the subsequent crop type (e.g. annual vs. perennial crops) that will replace the vegetation with natural BNI capacity, which in the case of perennial plantations remains unexplored until now. Hypothetical scenarios have been modeled to explain the residual BNI persistence depending on the type of subsequent crop (Subbarao et al., 2012), but a paucity of factual field evidence on this subject persist at the moment.

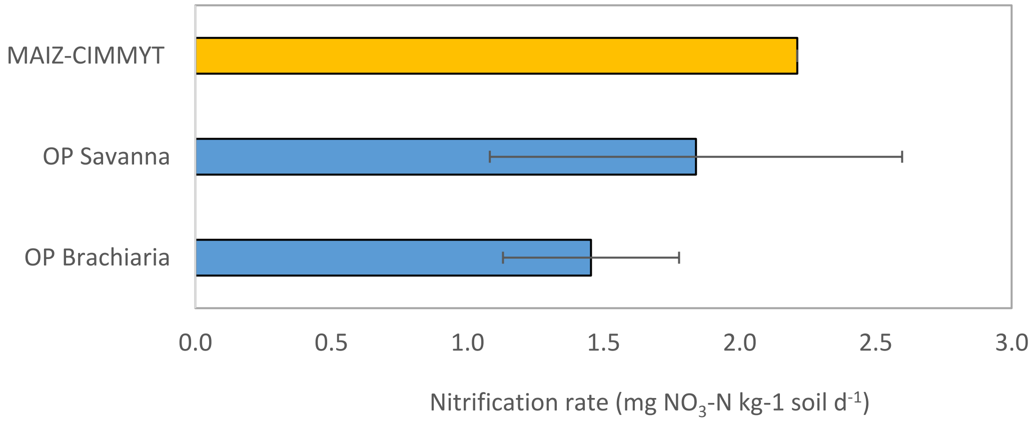


Fig. 2. Mean nitrification rates of OP plantations established on formerly savanna and Brachiaria pasture land across management zones (W, F, S and H) and plantation age (4- and 8-yr old). Horizontal lines represent SE. N= 5 and 4 for Brachiaria and savanna derived sites, respectively

Nitrification rate values differed among the four management zones of OP plantations in the following order: W > S > H > F. Only the W samples had greater nitrification rates compared to the Maiz-CIMMYT soil. The presumably higher C content and N inputs in organic forms in the F management zone samples could have altered the C:N ratio and this resulted in less NO_3 production. Likewise, the continuous input of exogenous N inputs via fertilizers in the W samples could have increased N mineralization. These findings suggest that management practices can also impact in different forms legacy BNI capacity.

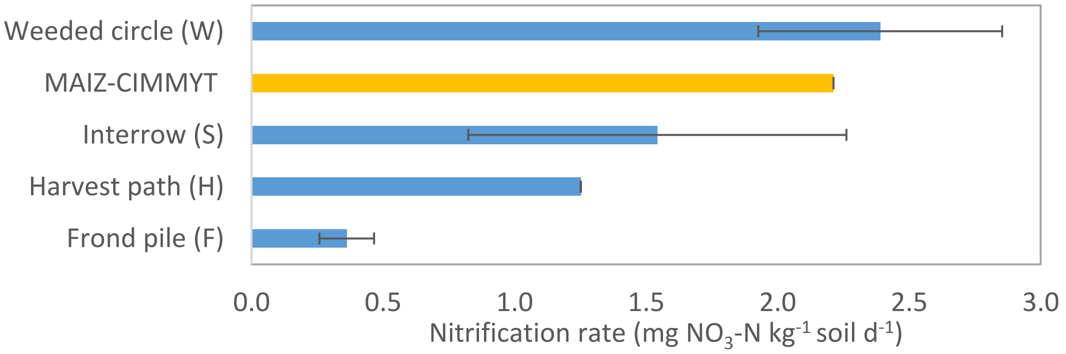


Fig. 3. Mean nitrification rates of OP plantations and Maiz-CIMMYT sample. Values are grouped by management zones (W, S, F and H) found in OP plantations across plantation age (4- and 8-yr old) and previous vegetation cover (savanna and Bachiaria pasture). Horizontal lines represent SE. N= 4, 2 and 2 for W, S and F management zones.

The wide variability found in nitrification rate values between OP management zones, plantations ages, previous vegetation covers and presence of different leguminous cover crop species provide an initial framework to develop a series of studies to assess the impacts of land-use change not only on BNI-capacity, but also on nitrogen cycling in low-fertility acidic savanna soils. The incubation procedure carried out in this study is part of a series of other experiments (including the use of molecular techniques that aim to quantify the copy number of *amoA* genes and a bioassay to detect BNI capacity in soil-plant systems) that need to be conducted to determine with convincing and robust evidence a regulation of nitrification in savannas/pasture former areas converted into OP plantations.

Future research directions should move toward gaining a better understanding of the persistence and effectiveness of natural nitrification inhibitors as a result of land-use conversion. Furthermore, the inherent high spatial variability that is characteristic of OP plantations provides a great deal of opportunities to investigate how field operations can affect N dynamics in the acidic savanna soils of Colombia.The effect of having leguminous cover crops in the traditional management of OP planation is equally relevant to study since it is well known that legumes can strongly influence nutrient cycling in a positive manner. Moreover, the interplay between their natural nitrogen fixing capacity and the persistence of nitrification inhibitors is subject of novel investigation. In this sense, there is almost no research done regarding the effects of this innovative OP/legume systems on oil palm trees nutrition and soil fertility. These preliminary results support a growing body of evidence that demonstrates that the maintenance of BNI-capacity for long periods of time or even permanently is plausible. This soil-plant trait could be key to enhance nutrient use efficiency and reduce N_2O emissions in agricultural areas where land is being converted from savanna/Brachiaria pasture into OP plantations and other crops.

Table 1. Detail of samples used for nitrification rate incubation

Sample #	Origin of the sample	Current land use	Previous land use	Planting year	Age categories (years old)	Origin of samples (Managment zones)[a]	Cover crops presence	Basal measurements	Technical replicates	Meaurements in time	Total amount of samples
1	Altillanura	Savanna 1	Savanna	~1960	>50	Transect	N/A	1	3	3	10
2	Altillanura	Brachiaria spp. 1[b]	Savanna	~1995	~20	Transect	N/A	1	3	3	10
3	Altillanura	Brachiaria spp. 2[c]	Savanna	~2000	~15	Transect	N/A	1	3	3	10
4	Altillanura	Oil palm plantation 1	Savanna	2014	2	W	Kudzu	1	3	3	10
5	Altillanura	Oil palm plantation 1	Savanna	2014	2	S	Kudzu	1	3	3	10
6	Altillanura	Oil palm plantation 2	Savanna	2012	4	W	Desmodium	1	3	3	10
7	Altillanura	Oil palm plantation 2	Savanna	2012	4	S	Desmodium	1	3	3	10
8	Altillanura	Oil palm plantation 3	Savanna	2008	8	W	No	1	3	3	10
9	Altillanura	Oil palm plantation 3	Savanna	2008	8	F	No	1	3	3	10
10	Altillanura	Oil palm plantation 4	Brachiaria spp.	2008	8	W	Brachiaria in the H zone	1	3	3	10
11	Altillanura	Oil palm plantation 4	Brachiaria spp.	2008	8	F	Brachiaria in the H zone	1	3	3	10
12	Altillanura	Oil palm plantation 4	Brachiaria spp.	2008	8	H	Brachiaria in the H zone	1	3	3	10
13	Altillanura	Oil palm plantation 5	Brachiaria spp.	2012	4	W	Brachiaria mixed with kudzu	1	3	3	10
14	Altillanura	Oil palm plantation 5	Brachiaria spp.	2012	4	S	Brachiaria mixed with kudzu	1	3	3	10
15[d]	Corpoica La libertad	Maiz field	Brachiaria spp.	2006	NA	N/A	N/A	1	3	3	10
16[e]	Altillanura	Oil palm plantation 5	Brachiaria spp.	2012	4	W	Brachiaria mixed with kudzu	0	3	3	9

[a] W= weeded circle; F= frond piles; S= between palms; Transect= 10 sampling points taken along a transect at 10 m distance in north-south direction

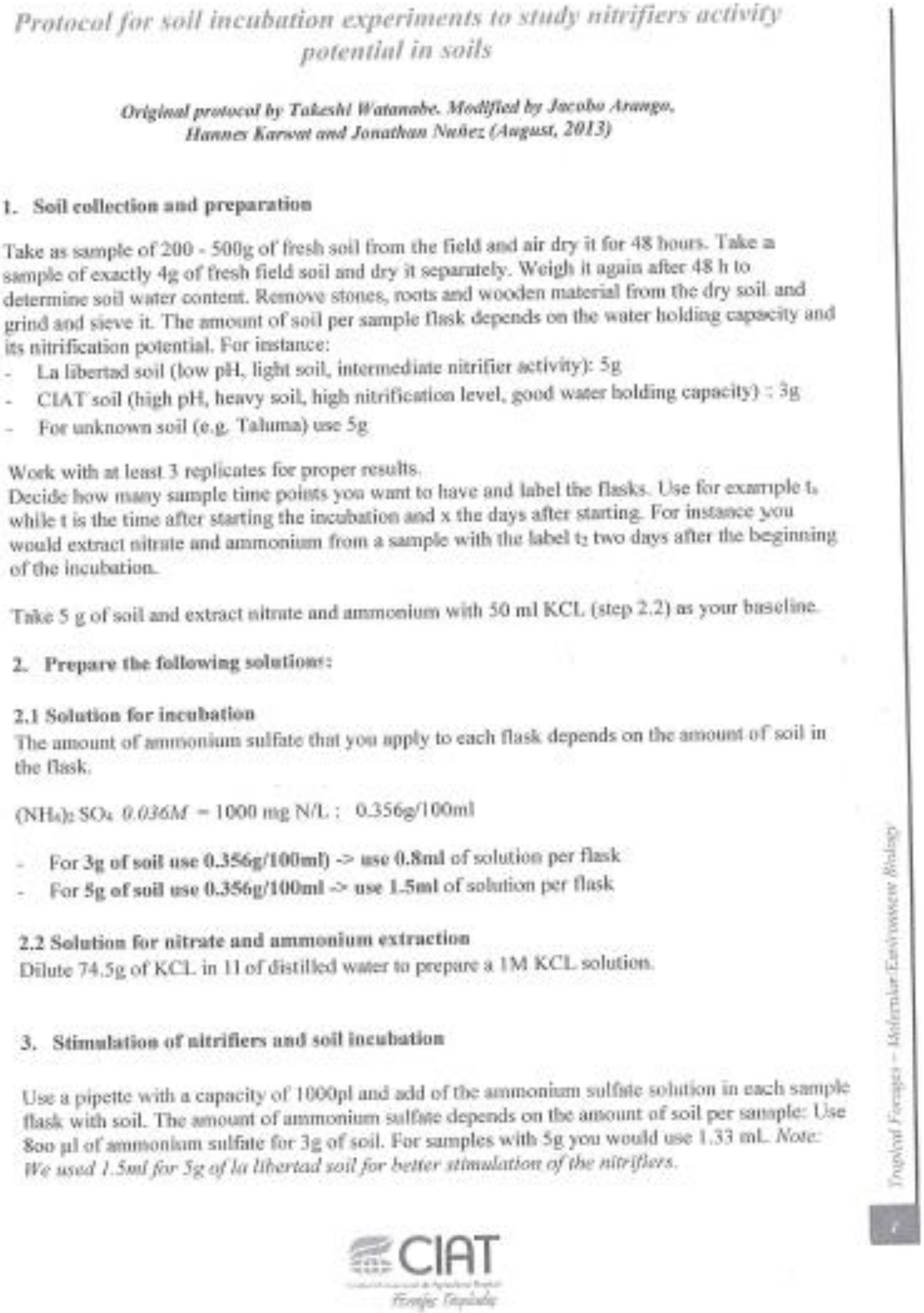
[b] Possibly Brachiaria decumbes

[c] possibly mulato hybrid

[d] Samples from a maize field at Corpoica La Libertad station

[e] Sample where the synthetic inhibitor DCD was added

Annex 1. CIAT protocol procedure to study Nitrifiers potential in soils



Protocol for soil incubation experiments to study nitrifiers activity potential in soils

Original protocol by Takeshi Watanabe. Modified by Jacobo Arango, Hannes Karwat and Jonathan Nuñez (August, 2013)

1. Soil collection and preparation

Take as sample of 200 - 500g of fresh soil from the field and air dry it for 48 hours. Take a sample of exactly 4g of fresh field soil and dry it separately. Weigh it again after 48 h to determine soil water content. Remove stones, roots and wooden material from the dry soil and grind and sieve it. The amount of soil per sample flask depends on the water holding capacity and its nitrification potential. For instance:

- La libertad soil (low pH, light soil, intermediate nitrifier activity): 5g
- CIAT soil (high pH, heavy soil, high nitrification level, good water holding capacity) : 3g
- For unknown soil (e.g. Taluma) use 5g

Work with at least 3 replicates for proper results.
Decide how many sample time points you want to have and label the flasks. Use for example t_x while t is the time after starting the incubation and x the days after starting. For instance you would extract nitrate and ammonium from a sample with the label t_2 two days after the beginning of the incubation.

Take 5 g of soil and extract nitrate and ammonium with 50 ml KCL (step 2.2) as your baseline.

2. Prepare the following solutions:

2.1 Solution for incubation

The amount of ammonium sulfate that you apply to each flask depends on the amount of soil in the flask.

$(NH_4)_2 SO_4$ *0.036M* = 1000 mg N/L : 0.356g/100ml

- For **3g of soil use 0.356g/100ml) -> use 0.8ml** of solution per flask
- For **5g of soil use 0.356g/100ml -> use 1.5ml** of solution per flask

2.2 Solution for nitrate and ammonium extraction

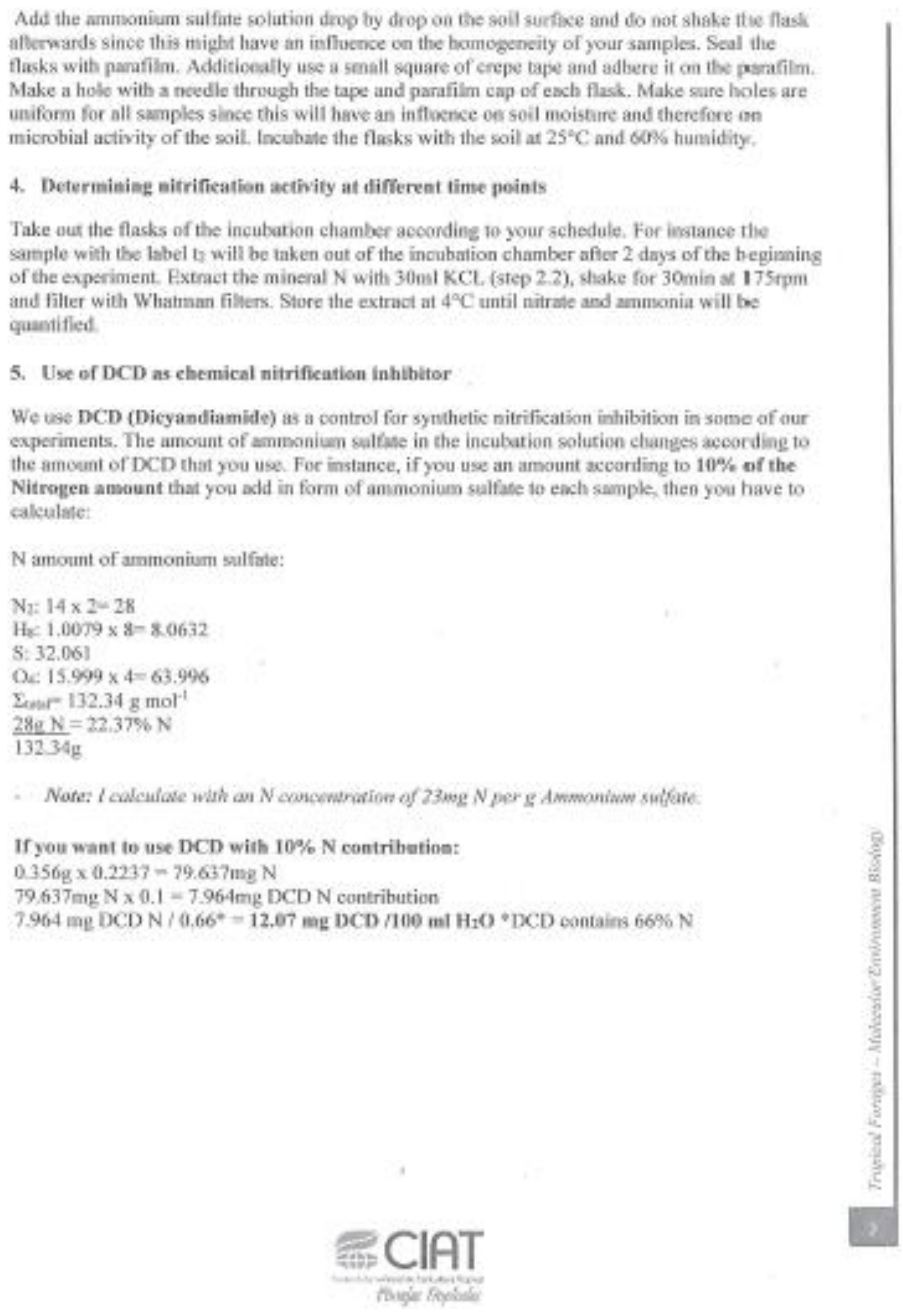
Dilute 74.5g of KCL in 1l of distilled water to prepare a 1M KCL solution.

3. Stimulation of nitrifiers and soil incubation

Use a pipette with a capacity of 1000pl and add of the ammonium sulfate solution in each sample flask with soil. The amount of ammonium sulfate depends on the amount of soil per sample: Use 8oo µl of ammonium sulfate for 3g of soil. For samples with 5g you would use 1.33 mL. *Note: We used 1.5ml for 5g of la libertad soil for better stimulation of the nitrifiers.*

Tropical Forages – Molecular Environment Biology

CIAT



Add the ammonium sulfate solution drop by drop on the soil surface and do not shake the flask afterwards since this might have an influence on the homogeneity of your samples. Seal the flasks with parafilm. Additionally use a small square of crepe tape and adhere it on the parafilm. Make a hole with a needle through the tape and parafilm cap of each flask. Make sure holes are uniform for all samples since this will have an influence on soil moisture and therefore on microbial activity of the soil. Incubate the flasks with the soil at 25°C and 60% humidity.

4. Determining nitrification activity at different time points

Take out the flasks of the incubation chamber according to your schedule. For instance the sample with the label t_2 will be taken out of the incubation chamber after 2 days of the beginning of the experiment. Extract the mineral N with 30ml KCL (step 2.2), shake for 30min at 175rpm and filter with Whatman filters. Store the extract at 4°C until nitrate and ammonia will be quantified.

5. Use of DCD as chemical nitrification inhibitor

We use **DCD (Dicyandiamide)** as a control for synthetic nitrification inhibition in some of our experiments. The amount of ammonium sulfate in the incubation solution changes according to the amount of DCD that you use. For instance, if you use an amount according to **10% of the Nitrogen amount** that you add in form of ammonium sulfate to each sample, then you have to calculate:

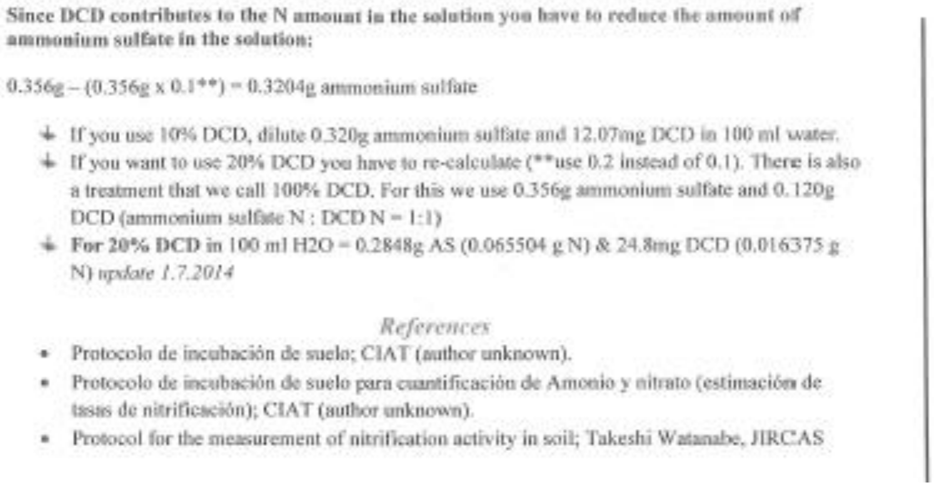
N amount of ammonium sulfate:

N_2: 14 x 2= 28
H_8: 1.0079 x 8= 8.0632
S: 32.061
O_4: 15.999 x 4= 63.996
Σ_{total}= 132.34 g mol^{-1}
$\frac{28g\ N}{132.34g}$ = 22.37% N

- *Note: I calculate with an N concentration of 23mg N per g Ammonium sulfate.*

If you want to use DCD with 10% N contribution:
0.356g x 0.2237 = 79.637mg N
79.637mg N x 0.1 = 7.964mg DCD N contribution
7.964 mg DCD N / 0.66* = **12.07 mg DCD /100 ml H_2O** *DCD contains 66% N

CIAT



Since DCD contributes to the N amount in the solution you have to reduce the amount of ammonium sulfate in the solution:

0.356g – (0.356g x 0.1**) = 0.3204g ammonium sulfate

- If you use 10% DCD, dilute 0.320g ammonium sulfate and 12.07mg DCD in 100 ml water.
- If you want to use 20% DCD you have to re-calculate (**use 0.2 instead of 0.1). There is also a treatment that we call 100% DCD. For this we use 0.356g ammonium sulfate and 0.120g DCD (ammonium sulfate N : DCD N = 1:1)
- **For 20% DCD** in 100 ml H2O = 0.2848g AS (0.065504 g N) & 24.8mg DCD (0.016375 g N) *update 1.7.2014*

References

- Protocolo de incubación de suelo; CIAT (author unknown).
- Protocolo de incubación de suelo para cuantificación de Amonio y nitrato (estimación de tasas de nitrificación); CIAT (author unknown).
- Protocol for the measurement of nitrification activity in soil; Takeshi Watanabe, JIRCAS

Annex 2. Photos

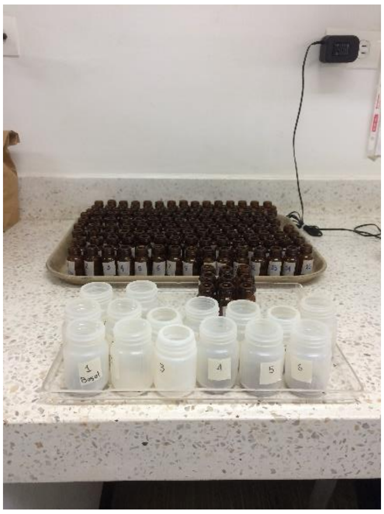


Photo 1. Complete set of samples to be analyzed (159 vials)

Photo 2. Set of vials that make one sample (10 vials)

Photo 3. Full set of samples in the incubator at 25°C

Photo 4. KCl extractions

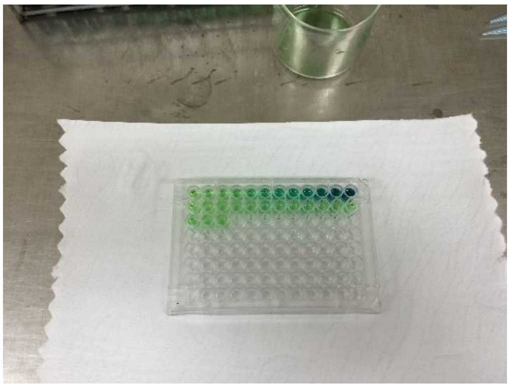


Photo 5. Microplate for NO_3 analyses

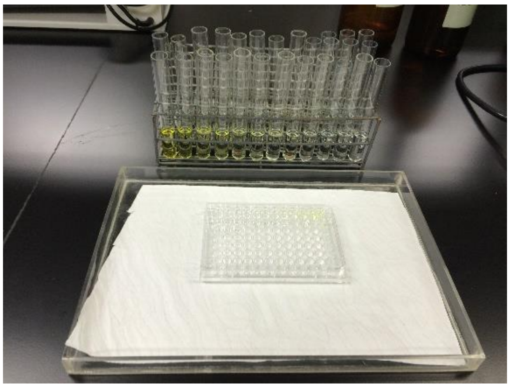


Photo 6. Microplate for NH_4 analyses

Photo 7. Young OP plantation with Kudzu as cover crop

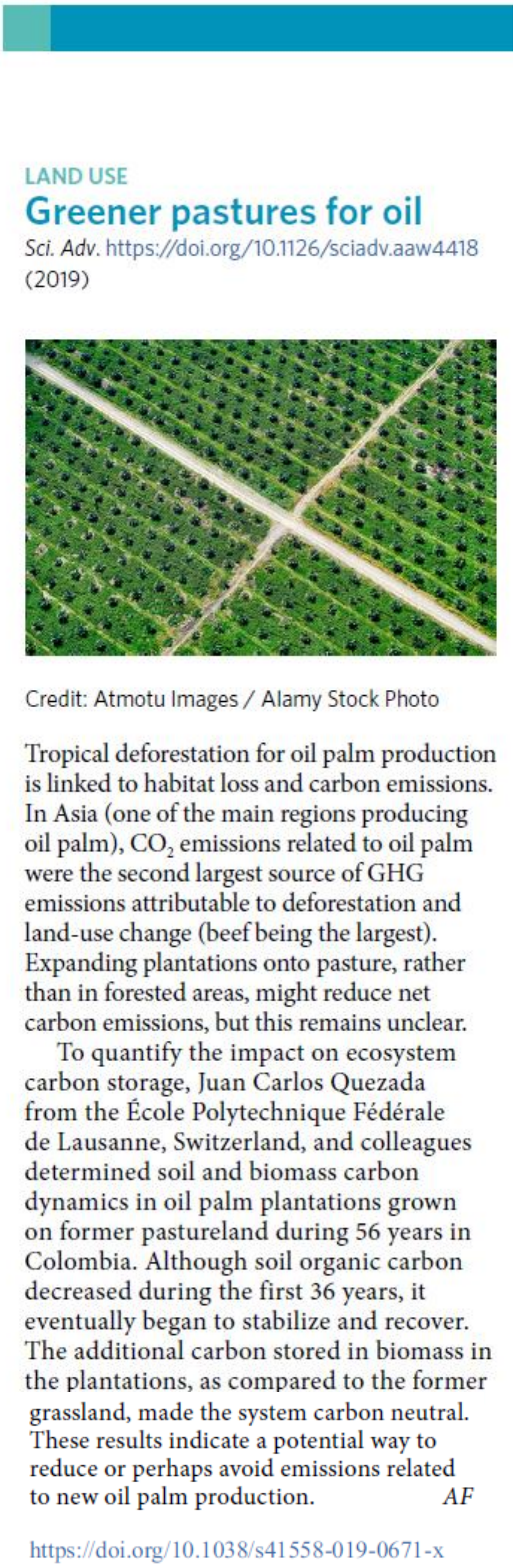
Photo 8. Semi-natural savanna in Altillanura

Photo 9 and 10. Brachiaria persisting in a young OP plantation

Photo 11 and 12. Brachiaria persisting in a mature plantation and Centrosema spp. recently seeded as cover crop

Photo 13 and 14. The two sites sampled with different Brachiaria species

9.2 Appendix 2: Research Highlight in Nature Climate Change



LAND USE

Greener pastures for oil

Sci. Adv. https://doi.org/10.1126/sciadv.aaw4418 (2019)

Credit: Atmotu Images / Alamy Stock Photo

Tropical deforestation for oil palm production is linked to habitat loss and carbon emissions. In Asia (one of the main regions producing oil palm), CO_2 emissions related to oil palm were the second largest source of GHG emissions attributable to deforestation and land-use change (beef being the largest). Expanding plantations onto pasture, rather than in forested areas, might reduce net carbon emissions, but this remains unclear.

To quantify the impact on ecosystem carbon storage, Juan Carlos Quezada from the École Polytechnique Fédérale de Lausanne, Switzerland, and colleagues determined soil and biomass carbon dynamics in oil palm plantations grown on former pastureland during 56 years in Colombia. Although soil organic carbon decreased during the first 36 years, it eventually began to stabilize and recover. The additional carbon stored in biomass in the plantations, as compared to the former grassland, made the system carbon neutral. These results indicate a potential way to reduce or perhaps avoid emissions related to new oil palm production. *AF*

https://doi.org/10.1038/s41558-019-0671-x

9.3 Appendix 3: Simple Density Fractionation

August, 2017

9.3.1 Introduction

This work performed at the University of Lausanne in collaboration with the Biogeosciences Lab under the supervision of Stephanie Grand. Topsoil (0-10 cm) soil samples from the Long-term chronosequence (pasture conversion into OP, Manuscript 1) were analyzed. In general , samples were high in Al, Fe, kaolinite with loamy texture, mostly single grained and ~10% angular aggregates. The C content of samples was of about 3% SOC and pH ranged between 4.5-5. Soils were of natural low fertility with low base saturation, available P and moderate CEC. This work provided the preliminary results to perform the work in Manuscript 4. The results at the end of this section were presented in a different report in order to obtain some funding to perform more in-depth fractionation work at the Thunen Institute in Germany under the supervision of Christopher Poeplau and Axel Don. Finally, 5,000 CHF were granted by the Doctoral Program in Civil and Environmental Engineering (EDCE) at EPFL so that a part of the expenses generated in Manuscript 4 were covered by this PhD mobility Award.

9.3.2 Material and Methods

We aim to separate the following fractions: free light fraction (FLF), occluded light fraction (OcLF) and heavy fraction (HF). Figure 1 and 2, present the fractionation scheme used these three SOC fractions.

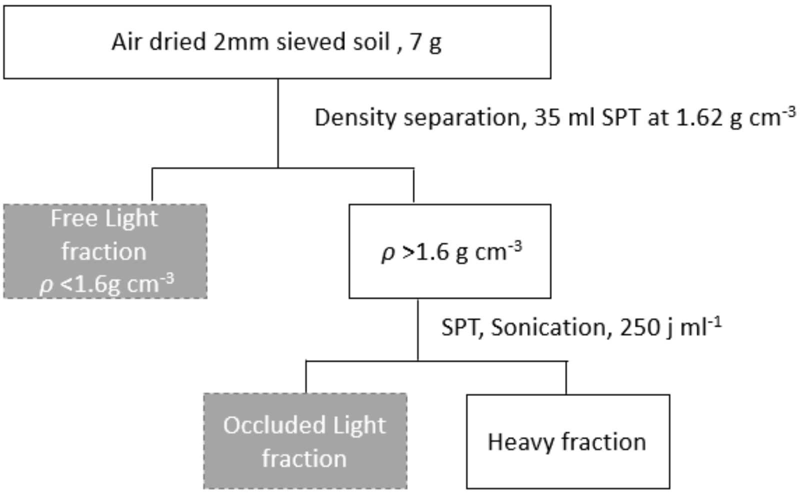
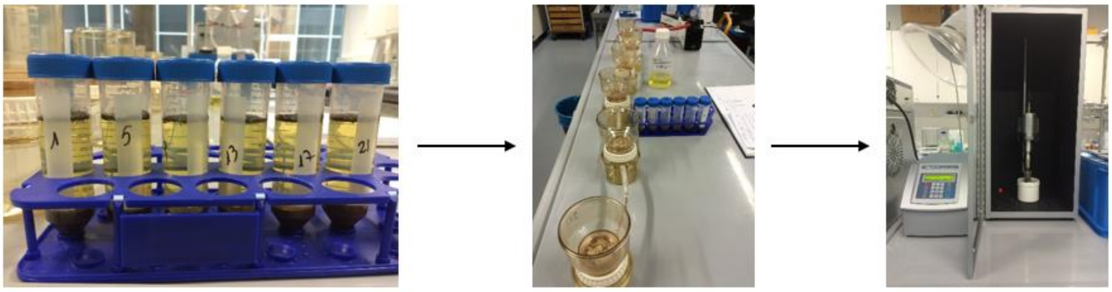


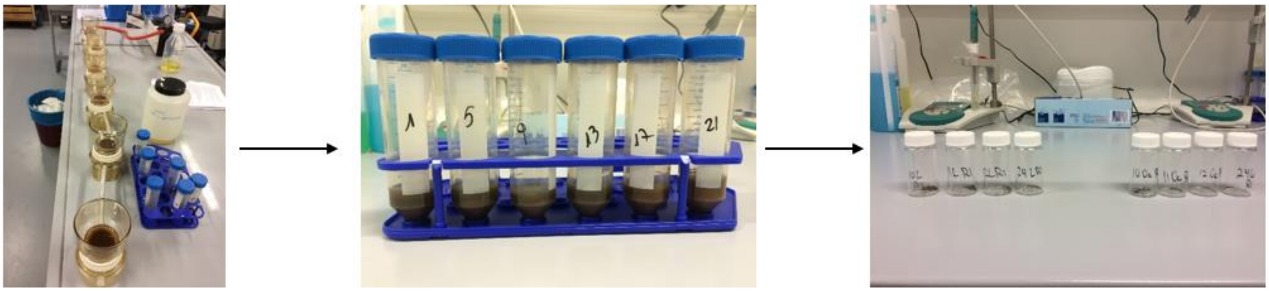
Figure 1. Fractionation scheme used to separate three SOC fractions.



Supernatant containing free light fraction $\rho < 1.6 g\ cm^{-3}$

Filtration line retaining free light fraction

Sonication at 250 J/ml to disrupt aggregates occluding particulate organic matter



Filtration line retaining occluded light fraction

Heavy fraction remaining in tubes after removing light and occluded light fractions

Light and occluded fractions stored in scintillation vials

Figure 2. Fractionation scheme used to separate three SOC fractions.

9.3.3 Results

As expected, replacement of pasture areas by OP plantations gradually reduced overall $\delta_{13}C$ values in the three separated SOC pools with time (from -14‰ to -28‰ in the 56 years plantation). Our preliminary results show that rates of isotopic changes in the three separated pools decreased in the following order: FLF>OcLF>HF (Fig. 1). No pasture-derived C was found in the LF fraction after 18 years of OP cultivation (δ13C ~28 ‰), indicating a very fast decay of pasture derived C in this fraction. Conversely, about half of total SOC was still pasture-derived in the HF after two OP rotation cycles (Fig. 1.). At all sites the bulk of SOC was associated with the HF (83 to 72%).

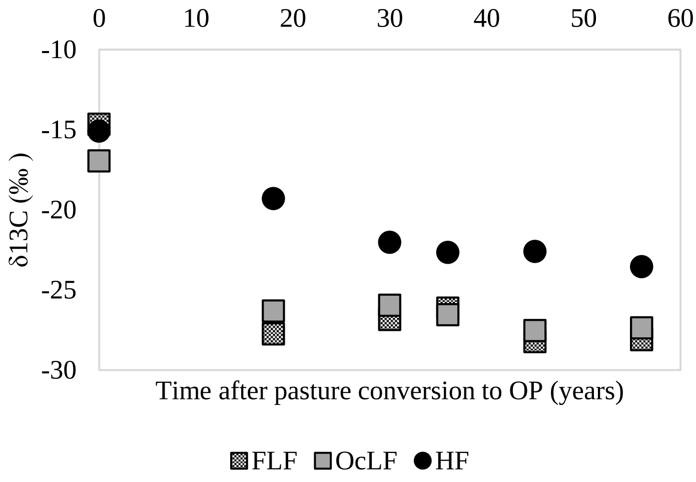


Fig.1. Changes in natural ^{13}C signature (0-10 cm depth) in three separated SOC fractions following pasture conversion into OP up to 56 years. Time 0 refers to one pasture site. Data corresponding to the subset of analyzed research sites.

The C content in the OcLF decreased sharply after plantation establishment, but it started to increase again after OP plantation renovation during the second cultivation cycle, indicating a long-term resilience of this fraction against LUC (Fig. 2.). Apparently, enhanced physical protection of SOM is increased by the perennial life cycle of OP plantations where tillage is performed only once (at plantation establishment), and a significant pulse of OM input following plantation renovation that is provided for the second rotation cycle. Nevertheless, whole soil C stocks were depleted by almost 50% after 56 years of pasture conversion (Manuscript 1). Our results suggest that despite enhanced physical protection of SOC in aggregates, which can increase MRT of SOM, after plantation renovation, OP cultivation following pasture conversion reduces SOC stocks over two rotation cycles. Reductions in overall SOC stocks was directly correlated to significant reductions in the C content of the HF pool that contained most of the total SOC.

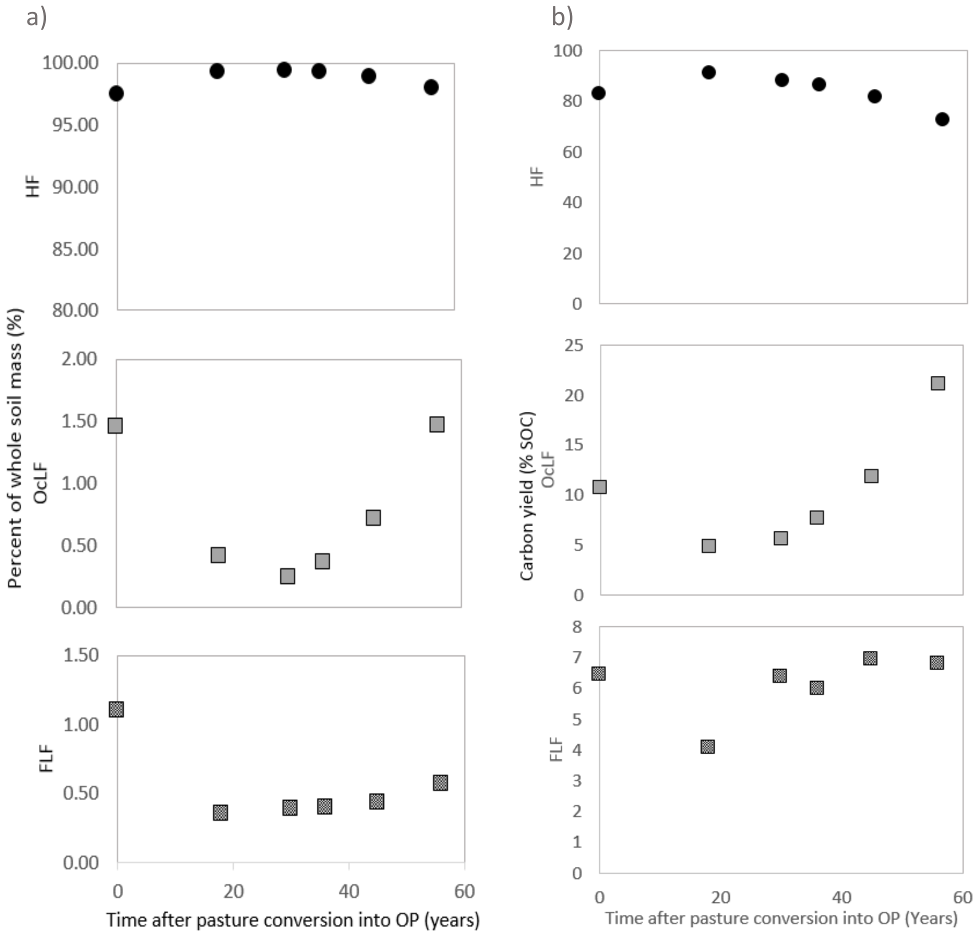


Fig.2. a) Share of mass in whole soil mass; b) Carbon yield (% total SOC) in three separated SOC pools. Data corresponding to the subset of analyzed research sites (6).

9.4 Appendix 4: Manuscript 5: Drought-induced decline of productivity in the dominant grassland species Lolium perenne L. depends on soil type and prevailing climatic conditions

Alexandre Buttler, Pierre Mariotte, Marco Meisser, Thomas Guillaume, Constant Signarbieux, Amarante Vitra, Sara Preux, Géraldine Mercier, Juan Quezada, Luca Bragazza, Konstantin Gavazov

Status: Published in Soil Biology and Biogeochemistry, May 2019

9.4.1 Abstract

Severe constraints on grasslands productivity, ecosystem functions, goods and services are expected to result from projected warming and drought scenarios under climate change. Negative effects on vegetation can be mediated via soil fertility and water holding capacity, though specific mechanisms are fairly complex to generalise. In field drought experiments, it can be difficult to disentangle a drought effect *per se* from potential confounding effects related to vegetation or soil type, both varying along with climate. Furthermore, there is the need to distinguish the long-term responses of vegetation and soil to gradual climate shift from responses to extreme and stochastic climatic events. Here we address these limitations by means of a factorial experiment using a single dominant grassland species (the perennial ryegrass *Lolium perenne* L.) grown as a phytometer on two soils types with contrasted physicochemical characteristics, placed at two elevation sites along a climatic gradient, and exposed to early or late-season drought during the plant growing season.

Warmer site conditions and reduced precipitation along the elevational gradient affected biogeochemistry and plant productivity more than the drought treatments alone, despite the similar magnitude in volumetric soil moisture reduction. Soil type, as defined here by its organic matter content (SOM), modulated the drought response in relation to local site climatic conditions and, through changes in microbial biomass and activity, determined the seasonal above and belowground productivity of *L. perenne*. More specifically, our combined uni- and multivariate analyses demonstrate that microbes in a loamy soil with low SOM are strongly responsive to change in climate, as indicated by a simultaneous increase in their C,N,P pools at high elevation with cooler temperatures and wetter soils. Contrastingly, microbes in a clay-loam soil with high SOM are mainly sensitive to temperature, as indicated by a strong increase in microbial biomass under warmer temperatures at low elevation and a concomitant increase in C:N, C:P and N:P ratios. High SOM promoted a better annual yield of the phytometer grass under warmer climate and the effect of drought on productivity was transient. In contrast, low SOM reduced cumulative yield under warmer conditions and root production strongly decreased, enduring a lasting drought effect. Microbes in soils with high organic matter remained more active during warmer and drier conditions, ensuring soil fertility and stimulating a higher overall plant nutrient availability and productivity.

Our study highlights the important role of soil type for grassland responses to both stochastic climatic extremes and long-term climate change. Management practices enhancing SOM accumulation via organic residue incorporation seem a promising way to mitigate the effects of increased temperature and drought on plants and soil microbes alike promoting thereby a sustainable ecosystem functioning.

Keywords: climate change, rain shelter, microbial biomass, microbial activity, organic matter, fertility

9.4.2 Introduction

Along with the pressure of climate change, grasslands are challenged to meet the growing demand for ecosystem services and for providing human goods (EEA, 2012). Under sufficient water and nutrient supply, grassland productivity could benefit from future climatic scenarios, i.e. higher CO_2 concentrations, higher temperatures and longer vegetation periods (Lüscher et al. 2005). However, sufficient water availability during the vegetation growing season might be at stake under certain future climatic predictions. For central Europe, for example, it is predicted that the occurrence of dry summers will increase by the year 2050 (IPCC 2013) and that extreme drought events could become more frequent and intense in response to climate change (Ciais et al., 2005). In the western part of Switzerland, extreme summer droughts are predicted to cause 70% decrease in precipitations by the end of the 21th century (CH2011, 2011). Under such projected water deficits, we can then expect severe constraints on grassland productivity (Smith, 2011) as well as negative effects on other ecosystem functions, goods and services such as soil fertility, biodiversity and carbon (C) storage (de Vries et al., 2012, Jentsch et al., 2011).

The response of grasslands to drought and the degree to which ecosystem services are affected depend on the complex interactions among different factors involving plant community diversity, soil properties, climatic conditions and land management (see for example Deléglise et al. 2015, Thébault et al. 2014). Furthermore, duration, intensity and timing of the drought, as well as the frequency of rainfall events are all very important factors influencing the response of grassland communities to drought (Chou et al., 2008, Didiano et al. 2016). For example, using a mesocosm experiment, De Boeck et al. (2011) found that season of drought had a large effect on the magnitude of reduction in plant biomass and growth, with early summer drought having the largest impact followed by spring and then autumn droughts. This is likely because the early summer is a time of rapid plant development with leaf area at its peak and high temperatures favouring transpiration.

Higher growing-season temperatures and more frequent occurrence of drought periods have the capacity to alter C and nutrient cycling at ecosystem level, with a potential for significant plant–soil feedback (Melillo et al., 2002). Higher temperatures may lead to enhanced C losses from soils (Bond-Lamberty and Thomson, 2010) and faster nutrient cycling (Ineson et al., 1998), yet these increases may be offset, or even reversed, by reduced soil moisture (Davidson and Janssens, 2006). Drought stress and, more generally, lower soil moisture can reduce plant nutrient uptake by decreasing nutrient mineralization (Fierer and Schimel, 2002), nutrient diffusion and mass flow in soil (Chapin, 1991). When drought stress is followed by rewetting, this often results in enhanced mineralization (Canarini and Dijkstra, 2015), which has been attributed to nutrient release from dead microbial biomass (Borken and Matzner, 2009), or the decay of fine roots that have accumulated during the drying period (Sanaullah et al. 2012). Duration and intensity of drought, as well as frequency of drought-rewetting cycles will thus determine the net effect of drought stress on nutrient supply and soil organic C storage (He and Dijkstra, 2014). Therefore, while the ecophysiological response of plants to drought stress is rapid, soil fertility might respond differently and in a more complex way because of potential buffering capacities (Fridley et al., 2011).

Elevation gradients within a confined geographical region can be conceived as a natural climatic experiment where ecosystem processes can be primarily attributed to variation in temperature and precipitation. Soil monolith transplantation from high to low elevations has been shown to effectively simulate climate change (see for example Link et al., 2003; Mills et al., 2014), including a combined effect of temperature and precipitation. Alternatively, rain shelter experiments in grasslands have been increasingly used to single out drought effects. For example, Gilgen and Buchmann (2009) applied rain shelters to three grasslands at different elevations in Switzerland showing that there is not a general drought response, due to the different grassland types and associated plant functional types, local climate conditions and soil types. Such “space-for-time” substitutions make it difficult to disentangle the drought effect *per se* from potential confounding effects related to vegetation or soil type, which also vary along with climate. Another difficulty is disentangling long-term climate shifts (interpreted from elevation gradient analysis) from short-term climatic extremes (interpreted from drought treatments).

In an attempt to control for these confounding effects, we performed a pot experiment using two types of soils with different soil organic matter content, i.e. a loamy low-organic and a clay-loam high-organic soil, which were placed at two sites with different climatic conditions, and were further subjected to the effects of early and late-season droughts during the plant growing season. Perennial ryegrass (*Lolium perenne* L.) was grown in the pots as a phytometer since it is one of the most important forage grasses in Europe, rendering high yields and nutritional value. Moreover, because it has no distinct tolerance to drought, this forage species is likely to be particularly affected by climate change (Westermeier et al., 2016). With respect to plant productivity, we hypothesized that: (i) late-season drought has a stronger negative impact than early season drought, but that local climatic conditions interact with the timing in determining the direction and magnitude of the drought effect; (ii) long-term warmer and drier conditions at low elevation have a stronger effect on plant growth than short-term droughts, due to a combined reduction in assimilation capacity and in soil microbial activity and bioavailability of nutrients. Furthermore, with respect to soil properties, we hypothesized that (iii) drought effects on plant productivity are tempered in organic matter rich soils due to a higher ability to retain soil moisture, and (iv) higher soil organic matter content warrants positive effects of warmer climate in increasing microbial C, N and P pools, rendering these soils less prone to drought stress.

9.4.3 Materials and methods

Experimental design

The pot experiment took place within an existing rainout shelter experiment, which was installed in two permanent grasslands (sites) of Swiss Jura mountains in 2015-2016. One site was located in Chéserex (N 46°24’, E 6°10’) at 540 m a.s.l., representing conditions of low elevation (thereafter named “low site”), whereas the second site was located in St-George (N 46°30’, E 6°15’) at 945 m a.s.l representing conditions of high elevation (“high site”). Mean annual temperatures for the low and high sites were 10.4 and 7.6 °C, respectively, and mean annual precipitation were 1050 and 1290 mm, respectively. In this region, grasslands are highly productive (9–12 Mg DM ha^{-1} yr^{-1}) and are regularly grazed by cattle or mowed for haymaking. The dominant plant species are the same in both sites: *Lolium perenne* L., *Trifolium repens* L., *Poa trivialis* L., *Poa pratensis* L., *Dactylis glomerata* L., *Phleum pratense* L. and *Taraxacum officinale* (L.) Weber ex F.H. Wigg.

Briefly, the rainout shelter experiment which was used for the pot experiment consisted in a complete randomized block design with five replicates (rain shelters of 12.0 x 6.4 m) set up in both sites on a grassland surface of approximatively 1 ha. Drought was simulated in plots of 4.5 m^2 (0.9 m x 5 m) within the rain shelters which included also the control plots. The periods of water stress were based on the seasonal dynamic of the growth (Figure 1), either during the peak of growing season (treatment of early-season drought) or after the peak of growing season (treatment of late-season drought) for a period of 8 weeks. Based on the last 30-year precipitation average at each site, during a period of two months (see Table 1) control plots received 100% of precipitation whereas drought plots received 30% of precipitation simulating the periods of water stress (i.e. early-season and late-season drought). Before and after the period of water stress, drought plots received similar precipitation to the control plots (C). Watering was done twice a week by means of a flow controller and a custom-made watering stick, which was moved along the plots, thus mimicking a regular rain. The climatic conditions measured by a meteorological station installed at each site during the three periods of 8 weeks are given in Table 1. Mean vapor pressure deficit (VPD) was calculated for these periods as well. VPD is a measure of the drying power of air, therefore it directly influences the plant hydraulic capacity and, in turn, may impact rates of growth and overall plant health. VPD is calculated based on air relative humidity and air temperature as the difference between saturation and actual vapor pressure of air for a given temperature. A VPD threshold of 1.5 kPa was chosen to represent the approximate value above which stomata close under extremely dry air for most plant species (Sangines et al., 2018). This experimental design would allow disentangling long-term climate shift (site effect), from short time climate extreme (drought treatment effect).

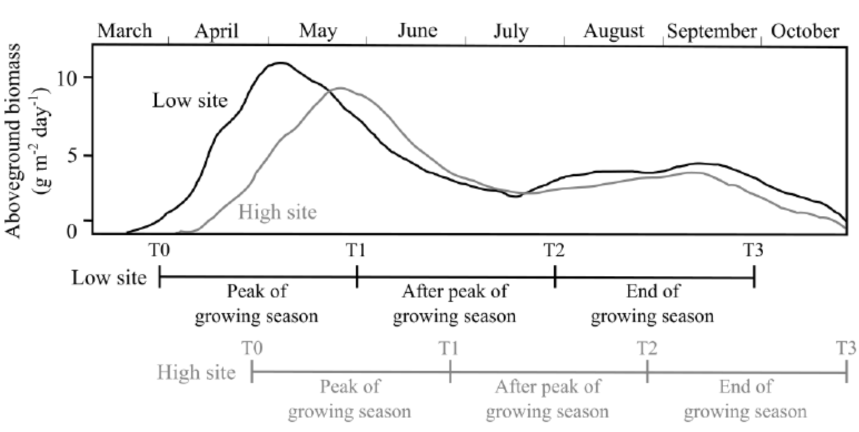


Fig. 1. Scheme of the seasonal dynamic of grassland biomass production for the two sites (unpublished data available from Agroscope institute). The different periods of plant growth along the season (each lasting for 8 weeks) are represented below the graph (Peak of growing season, After peak of growing season and End of growing season) with their respective sampling times (T1, T2 and T3). The ‘peak of growing season’ period has been centred on the peak of the vegetation growth curve and determined the beginning of the experiment at each site. T0 corresponds to the sampling time for initial conditions. The growth curve, periods and sampling times are drawn in black for the low site (540m a.s.l.) and grey for the high site (945m a.s.l.). Early-season drought was applied during the peak of growing season, while late season drought was applied after the peak of growing season.

The use of pots allowed to integrate in the experimental design the effect of soil type in 2016. Soils have been collected in close proximity to each shelter and selected so as to be representative of two contrasted features (e.g. fertility, SOM) of the same dominant soil at these altitudes, which is a cambisol. Soils had similar grassland species. At both site we took the top soil (0-30 cm). The low site had a low-organic matter content (thereafter named “loamy low-organic” or ”LOM”), and the high site had a higher organic matter content (thereafter named “clay-loam high-organic” or “HOM”). Besides the difference in organic matter content, these two soils differ also in their particle size fractions (determined on air-dried soils by sieving and the hydrometer method after removal of organic fraction with 30% H2O2 – Guy and Bauder, 1986), in their fertility and pH, being the HOM soil characterized by a finer texture and higher values of inorganic N, available P and pH as compared to the LOM soil (Table 2 and Supplementary table S1). Available water for plants in the soil (%) is comparable between both soils and it is calculated as difference in water content at pF=4.2 and pF=2.5 measured by means of a pressure plate (N=10). Water content ranged between 34% and 33% at pF=2.5 (field capacity), and between 18% and 19% at pF=4.2 (permanent wilting point) for LOM and HOM soil, respectively.

In January 2016, both soils were collected up to a depth of 30 cm, larger stones and coarse organic residues removed, then used to fill pots of 9.3 l volume (diameter 20 cm, height 24.3 cm), which were stored under a roof at ambient winter temperature. In parallel, in a glasshouse under optimal growth conditions, shallow (3 cm) turfs of same diameter as the pots were prepared using each of the soils and sawn with the phytometer *Lolium perenne* L. (variety Salamandra 4n, Schweizer and Steffen, Switzerland) at a density of 0.7 g of seeds per pot. Following germination, the *Lolium* turfs were placed on top of pots, fertilised with a solution of N 27.5% (as ammonium nitrate), corresponding to 30 kg N ha^{-1}, and kept cool prior to their transfer to the field. This initial fertilization simulates a field common practice at the time of sowing (or after plant emergence). Another reason to apply this fertilizer was to get rapidly a dense plant cover and to standardize the initial plant available nutrient content in the two soils so as to avoid confounding effects at the start of the experiment. Altogether, the experimental design consisted in two sites (low *vs* high site), three drought treatments (plots: control, early-season and late-season droughts), two soil types (pots: loamy low-organic-LOM vs clay-loam high organic-HOM) and five replicates (rain shelters). In total there were 60 pots.

By end of March, once the morning frost events ceased at each site, the pots were transferred to the rain shelters. The timing of the entire procedure was calculated so as to have a delay of about 2-3 weeks between the placement in the low and high sites, and to match the vegetation seasonality at these two sites (Figure 1). In each recipient plot, two pits were prepared and randomly received a pot with each soil type. Pots received the same watering regime as the entire plot.

Table 1. Climate characteristics at the low and high elevation sites during the different periods of the experiment in 2016 and average 30-year rainfall, measured by a meteorological station installed at each site or nearby.

Site	Periods (8 weeks)	Average rainfall (1981-2010) (mm)	Mean air temperature (°C)	Mean air humidity (%)	Mean vapor pressure deficit (VPD) (kPa)	Number of hours with mean VPD > 1.5 kPa
Low site 540 m a.s.l	Early-season drought	143	10.5	66.3	0.48	28
	Late-season drought	154	18.0	69.4	0.78	215
	End of growing season	144	20.0	63.2	0.99	315
High site 945 m a.s.l	Early-season drought	234	9.9	80.0	0.27	3
	Late-season drought	213	16.7	72.1	0.59	48
	End of growing season	235	15.6	72.9	0.57	68

Precipitation applies for the conditions outside the rain shelters.

Table 2. Main physico-chemical characteristics of the soil (0-30 cm) used for the experiment.

Soil type	Total C (%)	Total N (%)	pH_{H2O}	Clay (%)	Silt (%)	Sand (%)	Inorganic N mg kg^{-1}	Available P mg kg^{-1}	Available water (%)
Loamy low-organic (LOM)	2.25	0.24	5.8	20	41	39	16	9	15.9
Clay-loam high-organic (HOM)	5.45	0.48	7.5	36	42	22	27	51	14.2

Soil properties

Soil samples were analysed at the final harvest (T3 – see Figure 1) for the top soil layer (0-12 cm) corresponding to the main rooting zone. The water content was determined gravimetrically by drying soil subsamples at 105 °C to a constant weight. Total carbon (C) and nitrogen (N) content in the bulk soil were analysed after ball milling. Soil C and N were determined under high-temperature oxidation using an elemental analyser (CE Instruments model NA2500 Nitrogen Carbon Analyser) and expressed as percent of dry soil weight. For the determination of microbial biomass carbon (Cmic) and nitrogen (Nmic), pairs of c. 5 g of fresh soil were weighed for each replicate and one sample from each pair was immediately extracted in 25 ml solution of 0.5 M K_2SO_4, whereas the other sample was put in a vacuum desiccator and subjected to chloroform vapour. After one day of fumigation, the fumigated soil sample was extracted with the same solution. Total C and N concentrations in the extracts of fumigated and non-fumigated samples were analysed by a TOC/TN analyser (Shimadzu TOC-V). To determine the soil available phosphorus (P) and the microbial phosphorus (Pmic), 3 g of fumigated and non-fumigated fresh soil was extracted with 40 ml of 0.5 M $NaHCO_3$ (Olsen method). Inorganic phosphorus content of the extracts was analysed by colorimetry using a spectrophotometer at 890 nm (Olsen et al. 1954). Microbial biomass C, N and P were estimated as the differences between the amounts of C, N and P after and before fumigation using an extractability factor of 0.45 for C (Vance et al., 1987), 0.54 for N (Brookes et al., 1985) and 0.4 for P (Brookes et al., 1982). Microbial biomass C, N and P and soil available P are expressed as $mg.kg^{-1}$ oven dry soil. Ammonium (N-NH4) and nitrate (N-NO3) concentrations were determined by continuous flow analyses using an automated analyser (SEAL AA3 HR Autoanalyser) after extraction of 5 g of fresh soil with 30 ml of 1 M KCl and the results expressed as $mg \cdot kg^{-1}$ oven dry soil.

Statistical analysis

Statistical analyses were performed for all measured variables either at the different sampling times T1, T2 and T3 (volumetric soil moisture, soil temperature, above-ground biomass of *L. perenne*, soil respiration) or only at the final harvest T3 (total root biomass of *L. perenne* and root biomass at two depths, microbial biomass C, N and P, soil NH_4, NO_3, extractable P and C/N, C/P and N/P ratios). Overall ANOVA tests (three-way) and tests for each soil separately (two-way) are given in the figures and in Supplementary table S2. In the two-way ANOVA, two factors, i.e. site (S: experimental sites at low and high elevation) and watering regime (D: control, early-season and late-season drought), as well as their interaction were tested with block as a random factor. In the three-way ANOVA, soil was given as additional factor (G: LOM and HOM soil types), including interactions. Redundancy analysis (RDA) was done on the multivariate data from the final harvest (standardized) for each soil type, with drought treatment (categorical variable), soil temperature and soil moisture given as explanatory variables. Canonical axes and explanatory variables were tested subsequently with permutation tests. Assumptions of normality and homoscedasticity of the residuals in all final models were verified visually using diagnostic plots, with log transformations being applied to data when necessary. All analyses were carried out using R 3.4.2 (R development core team, 2017).

9.4.4 Results

Soil temperature and moisture and air VPD

Considering the comparable range of plant water availability of the two soils and their moisture content at pF 2.5 and 4.2 (Table 1), it can be concluded that changes in measured soil volumetric water content (TDR) reflect well the plant available water. Over the growing season, both soil types placed at the high site retained on average, significantly more soil water compared to the low site (LOM soil: +9%, +39, +61, and HOM soil: +16%, +19% and +81%, at T1, T2 and T3, respectively; Supplementary figure S3). Both early-season and late-season droughts strongly and significantly decreased mean soil volumetric moisture at both sites (LOM soil: -30% and -60%, and HOM soil: -39% and -43% at T1 and T2, respectively). The overall wetter HOM than LOM soils (+ 7%) provided for their significantly ($P < 0.05$) higher moisture content at T2 during the second part of the growing season and buffered significantly the negative effect of the drought treatment (Soil×Drought, $P < 0.001$). With respect to temperature, during the early growing season there was no temperature difference between the two elevation sites for both soil types (on average 13.1°C) (Supplementary figure S4). Later in the season, however, both soils became increasingly warmer ($P < 0.001$) at the low site compared to the high site (for both LOM and HOM soils: +5.5 °C, +10.7 °C during late-season drought (T2) and end of growing season (T3), respectively). In addition, late-season drought (T2) induced slightly warmer conditions in both soil types (+ 0.9 °C; $P < 0.05$). HOM soils were on average slightly warmer than LOM soils (+0.2 °C; $P < 0.05$) during the entire growing season. The mean air temperature during the early-season drought period was +0.6 °C higher at the low site (Table 1), and during the late-season drought and the following two months it was +1.3 °C and +4.4 °C higher, respectively. However, the water stress for plants, as quantified by the number of hours with mean VPD > 1.5 kPa, increased at the low site during the late-season drought and the following two months.

Above and belowground dry matter yield

Yield of *Lolium perenne* was significantly different between the two soils at all sampling periods (overall +43% in HOM soils, $P<0.001$), with a strong interaction with site at the early-season drought (Figure 2). Yield did vary for the same soil type depending on site elevation and season. For both soil types, there was more yield at the low site at the end of the early-season drought period (Figure 2-T1; $P<0.001$), but the trend reversed later in the subsequent harvests as the production at the high site increased (Figure 2-T3; $P<0.05$ for LOM soil, $P<0.1$ for HOM soil). Drought had a weaker effect than site. Yield slightly decreased in both soil types during the late-season drought (Figure 2-T2, $P<0.05$), but this effect carried over until the end of the growing season only in the LOM soil at the high site (Figure 2-T3, interaction, $P<0.05$). With respect to the cumulative yield from the three harvests, it was different for both sites only in the HOM soil, with more yield at the low site (although marginally significant, $P<0.1$) and drought effect persisted only in the LOM soil ($P<0.01$). Interestingly, in the LOM soil, the early-season drought had a positive effect on aboveground plant production (Figure 2-T1; $P<0.01$).

Root production was also different depending on soil type (Supplementary figure 5: +15% total root biomass in HOM soils, $P<0.05$, with interaction with site). Total root biomass in the LOM soil was significantly lower at the low site ($P<0.05$), while in the HOM soil there was no difference between the two sites. Drought effect was only weakly significant, with interaction ($P<0.01$) showing an increase of roots in the LOM soil in the late-season drought at the high site (both at 0-12 and 12-22 cm depth – data not shown), while in the HOM soil this increase occurred only in the deeper soil layer (data not shown). In both soil types, only a minor fraction of total roots was found in the deeper layer (data not shown).

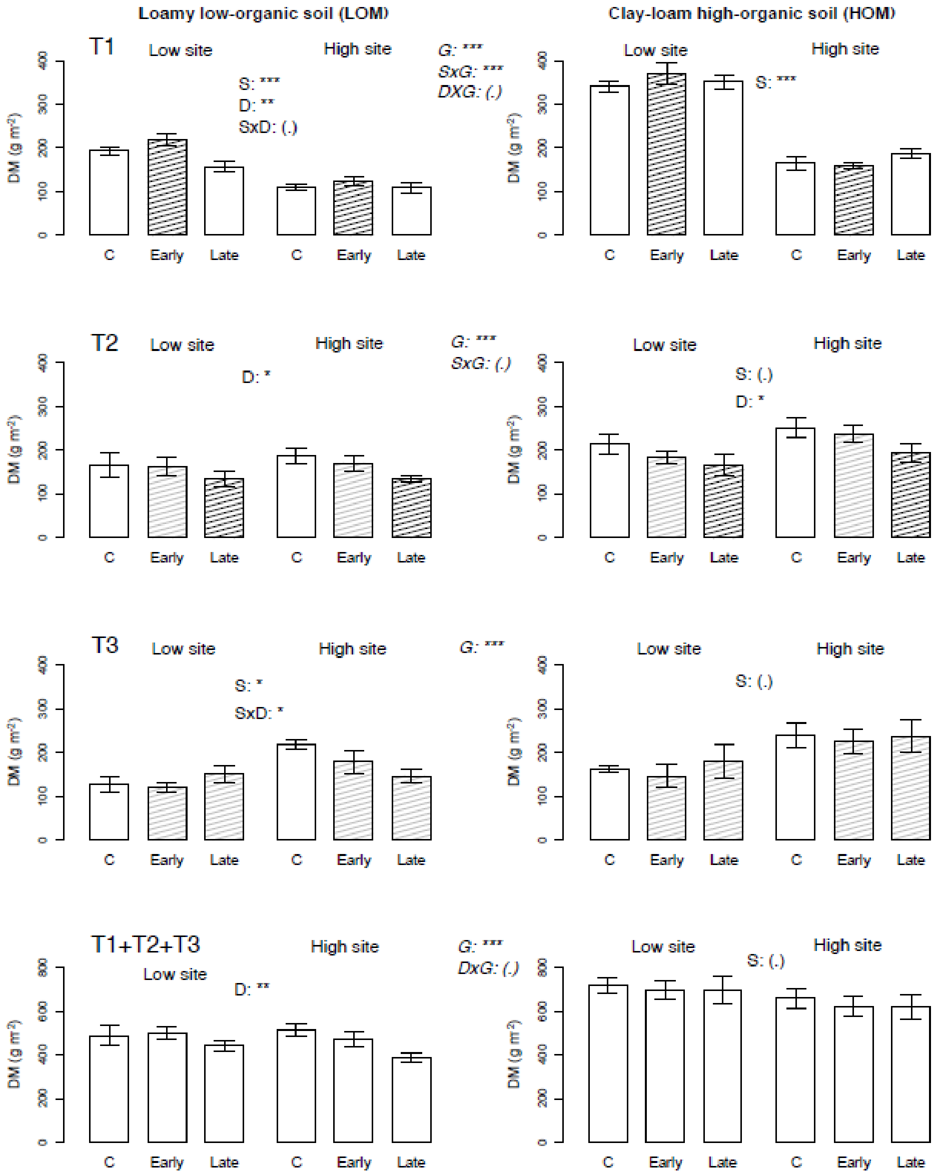


Fig. 2. Mean (±SE) above-ground biomass (N=5) of Lolium perenne after the early-season drought (T1), the late-season drought (T2) and at the end of the growing season (T3) for the two soil types and the two sites. The cumulative above-ground biomass is given at the bottom (T1+T2+T3). Both soil types were transplanted each in the two sites at low (540 m a.s.l) and high (945 m a.s.l) elevation. Black hatching highlights when pots were experiencing drought, either early-season (Early, T1) or late-season drought (Late, T2) treatment, while grey hatching highlights pots that were previously subjected to drought. No hatching corresponds to either control pots (C) or to pots which did not yet undergo the drought treatment (e.g. Late in T1). ANOVA tests are given for each soil with site effect (S), drought effect (D) and their interaction (SxD), and the overall test is given in italics with soil effect (G) and interaction with S and D. Significance levels are P < 0.001 (***), P < 0.01 (**), P < 0.05 (*) and P < 0.10 (.).

Soil respiration

Soil respiration was significantly different at all sampling periods depending on soil type ($P < 0.001$) and interactions with site were observed (Figure 3). Soils respired more at the low site at the end of the early-season (Figure 3-T1; +25%, $P < 0.05$ for HOM soil) and late-season (Figure 3-T2; LOM: +101% and HOM: +57%, $P<0.01$) drought periods, as well as at the end of the growing season (Figure 3-T3; LOM and HOM: +140%, $P<0.001$). Drought treatment effect on respiration was marked in HOM soils already during the early-season drought (Figure 3-T1; -26%, $P<0.01$), but late-season drought impacted negatively the soil respiration most significantly in the LOM soil (Figure 3-T2; -11% and -25% in early and late season drought, respectively, $P<0.05$). For the LOM soil a legacy effect was still visible at the end of the growing season (Figure 3-T3; -16% and -6% in early and late season drought, respectively, P <0.1).

Microbial biomass

Soil type significantly affected microbial biomass C and N ($P<0.05$) and there were highly significant interactions with site for all microbial biomass nutrients (Figure 4). In each soil type site effect was generally highly significant for microbial biomass C, N and P (Figure 4; $P<0.01$ with the only exception of microbial P in HOM soil). The microbial biomass C, N and P pools increased in the LOM soil when it was placed at the high site and in the HOM soil when it was placed at the low site. The drought effect was observed only for microbial biomass C in HOM soil (Figure 4a; $P<0.01$). The ratios of microbial C:N, C:P and N:P increased significantly in HOM soil when transplanted at the low site (Supplementary figure S6; $P<0.01$), while there were no significant changes in the LOM soil.

Soil available ammonium, nitrate and phosphorus

Soil type had an effect on soil nitrate ($P<0.05$) and available P ($P<0.001$), with strong interaction with site (Supplementary figure S7). LOM soil type did not show any significant pattern with respect to ammonium, nitrate and phosphorus. In HOM soil, nitrate showed a decrease under drought ($P<0.05$) and concentration of available P was significantly reduced at low site ($P< 0.001$), while ammonium was affected neither by site nor by drought.

Multivariate analysis

RDA scatter plots with LOM and HOM soils (Figure 5) shows that soil moisture and soil temperature are opposed along the first canonical axis, while drought treatment lies along axis 2. The general pattern was that total above-ground biomass was correlated to soil moisture, while soil respiration was correlated to temperature. Nitrate lies along axis 2 and it is more related to drought treatments, likewise the ratio above-belowground biomass. Most interestingly, microbial biomass N and P, as well as to some extent microbial biomass C, were related to soil moisture in the LOM soil, while in the HOM soil they were related to temperature. In HOM soil, microbial C:N, C:P and N:P ratios were strongly correlated to temperature, but not in the LOM.

9.4.5 Discussion

Seasonality of biomass productivity under different climatic conditions

In accordance with our first hypothesis, we found that local climatic conditions interact with the timing of drought occurrence to determine the direction and magnitude of shifts in plant productivity, based on the different stages of plant development. The higher mean air temperature at the low site during the early-season drought period favoured the growth of *L. perenne*, but later in the season the water stress for plants in relation to higher temperatures (e.g. VPD) increased drastically, causing a reduction in plant growth. Indeed, temperature has a beneficial role on the growth of temperate forage grasses, such as the perennial ryegrass *L. perenne* (Wingler and Hennessy, 2016).

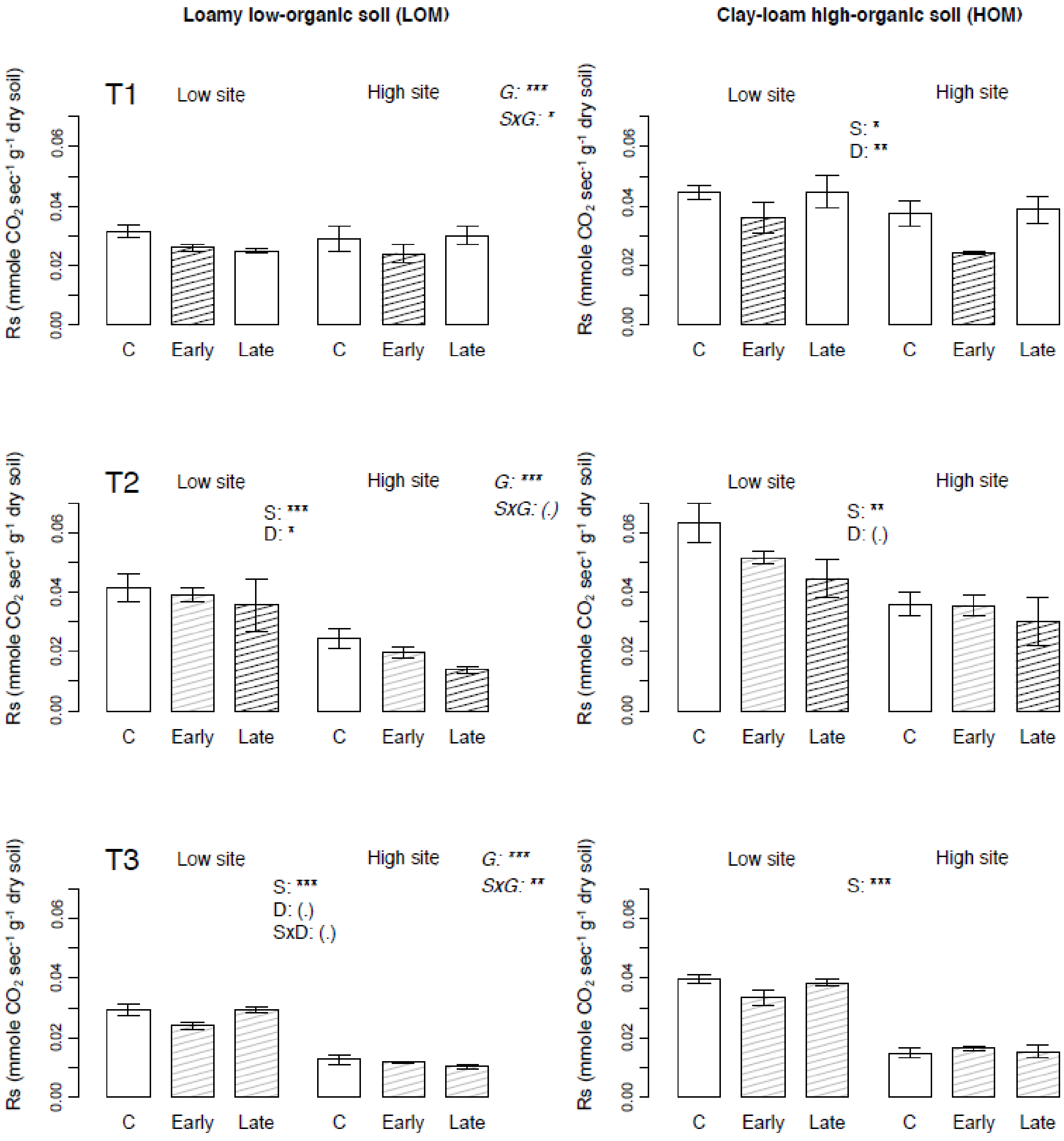


Fig. 3. Mean (±SE) soil respiration of the pots (N=5) after the early-season drought (T1), after the late-season drought (T2) and at the end of the growing season (T3) for the two soil types and the two sites. See Fig. 2 for additional information.

Nevertheless, the consequences of a prolonged water stress at landscape scale, as during the 2003 summer heat wave, were estimated in a 20-30% decrease of grassland production in Switzerland (Keller and Fuhrer, 2004). At plant species level, the most immediate effect of water stress is the limitation of plant growth through diminished leaf C fixation resulting from stomatal closure to prevent excessive transpiration (Signarbieux and Feller, 2012). Such growth reduction in response to experimental warming has also been observed in field-grown ryegrass (Nijs et al., 1996) and natural subalpine grassland communities (Gavazov et al. 2014, Mariotte et al. 2013). Furthermore, as hypothesized, late-season drought had a stronger negative impact than early-season drought, albeit this effect was inferior to the season-long combined temperature and precipitation shift along the elevational gradient, which determined the VPD for plant growth. It has been shown by Craine et al. (2012) that the inter-annual timing of climate variability is a factor determining the grassland productivity since, in addition to environmental conditions, developmental factors determine the growth of grasses (Hurtado-Uria et al., 2013). Noteworthy, the early-season drought seems to have promoted the growth of *L. perenne* in the LOM soil (Figure 2-T1), which can be explained by the positive effect of drier soils on temperature conduction (Seneviratne et al. 2006) at the onset of spring growth. When considering the cumulative aboveground yield of *L. perenne*, the drought had a lasting effect only in the LOM soil. Conversely, in the HOM soil, *L. perenne* could retain the advantage of an earlier onset of the growing season with warmer climate at the low site, due to better ability of the soil to retain moisture that mitigates the negative effects of drought.

With respect to the below-ground biomass, late-season drought, which had a strong effect on soil moisture, promoted root growth at depth, especially within the LOM soil at the high elevation site. It seems that under unfavourable climatic conditions, *L. perenne* was able to invest relatively more resources belowground when deeper water resources were available. Such root plasticity has also been reported in multispecies communities (Hernandez and Picon-Cochard, 2016), in which vertical root complementarity was beneficial for water and nutrient uptake. Although we did not specifically measure fine root production, we could speculate that the observed depth increase of seasonal root biomass at the high site could have been related to a change in relative root size and therefore modified the potential uptake of nitrogen (Clarkson et al., 1986) and thus the recovery in plant biomass production.

Effect on soil fertility and plant growth of long-term warmer and drier conditions vs short-term drought

In our experiment, the transplantation to a warmer and drier site had a stronger effect on soil fertility and plant growth as compared to the short-term drought treatments, despite a similar magnitude in soil moisture reduction. The soil microbial and chemical analyses were done only at the end of the growing season and therefore they do not strictly represent the response at the end of either of the two drought periods. However, they have the potential to highlight any drought legacy effect on microbial growth and nutrient mineralization. It seems that the legacy effect was absent, except for nitrate in the

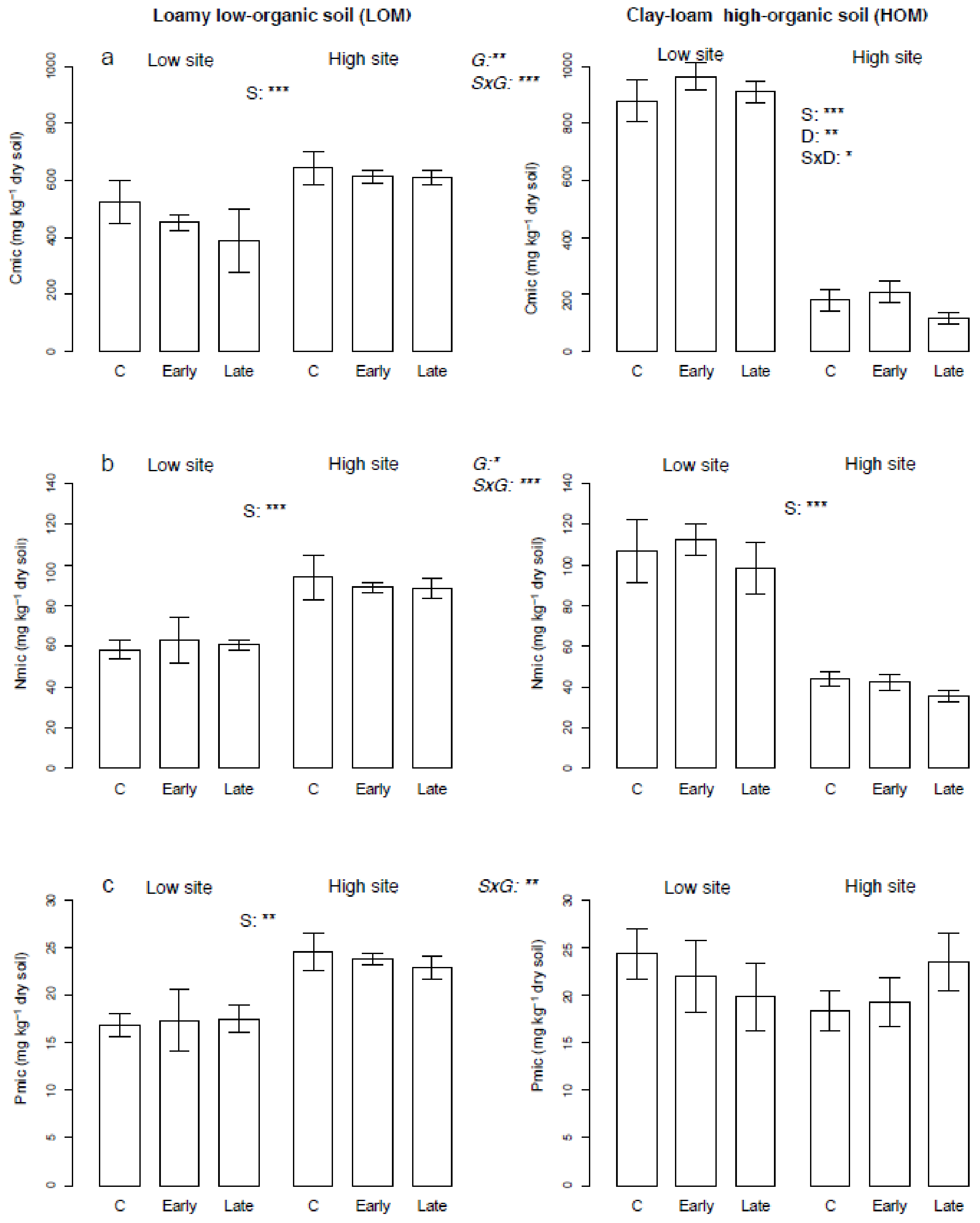


Fig. 4. Mean and SE (N=5) soil (0–12 cm) microbial C (a), N (b) and P (c) at final harvest (T3) in the control pots and in the pots with early-season and late-season drought treatments for the two soil types and the two sites. See Fig. 2 for additional information.

HOM soil, whose content was lower when soils had experienced a drought. In contrast, the warmer and drier soil conditions at the low site significantly reduced phosphorus availability and increased microbial C/P and N/P ratios in the HOM soil. Jupp and Newman (1987) found that the phosphorus uptake by *L. perenne* was reduced immediately after a period of drought due to reduced diffusion rate of phosphorus to the root surface in the drying soil, whereas after three weeks the recovery of uptake ability did occur. Although we could not evaluate the plant uptake and possible limitation in the absence of chemical analyses of plant biomass, the decrease in productivity at the end of the growing season (Figure 2-T3) for the warmer and drier HOM soils at the lower site seem to reflect this drop of extractable P. Indeed, in a meta-analysis, He and Dijkstra (2014) pointed to the increase in soil available N and a decrease in soil available P to explain the increase in plant N:P in response to drought. Overall our findings suggest that long-term (growing season) climatic conditions and the warming-induced drying of air and soil affect soil biological activity and nutrient bioavailability more than stochastic drought events and confirms our second hypothesis. Considering that soil legacy effects have been demonstrated after repeated drought stress (Legay et al., 2018), it is plausible that we only detected biogeochemical changes in response to the long-term climate shift along the elevation gradient.

Opposed soil temperature and soil moisture limitation for different soil types

Soils are diverse in mountainous regions, in particular for what concerns soil organic matter stocks and physicochemical characteristics, which makes predictions for their response to climate and land use change particularly difficult (Hagedorn et al., 2010). The two contrasting soil types responded differently to the two climate change treatments with respect to most of the measured physical, chemical and biological variables (Supplementary table S2). This was particularly noticeable for above-ground biomass and root biomass and it related also to the differences in fertility, in particular with respect to available N and P (Table 2). Microbial activity (here expressed as soil respiration and microbial biomass growth) differed significantly between the two soil types in response to a warmer and drier climate, likely due to inherent differences in the ability of the soil to retain moisture. Given that the rate of organic matter turnover in mountain grasslands is strongly dependent upon soil moisture only when temperature is not limiting microbial activity (Mills et al., 2014), it is not surprising that soil respiration rates in both soil types responded positively to the warmer conditions at low elevation. The soil moisture reduction induced by the drought treatment affected the respiration primarily in the LOM soil, especially during the late-season drought when this soil remained drier than HOM. Furthermore, in the LOM soil, the drought treatment had a legacy effect on soil respiration at the end of growing period (T3), two months after the late-season drought ceased, which indicates that soil microbial pool was affected beyond the drought period (Legay et al., 2018). The higher ability of HOM soil to retain moisture under field conditions is likely related to the combined effect of higher organic matter and clay contents, which together influence the soil structure. We presume that the soil structure of the HOM soil is such that it allows a better retention of precipitation water due to a lower rate of evaporation under drought, which could be explained by its pore's configuration. Overall, high SOM provides a buffered habitat for microbes (McGonicle and Turner, 2017; Xu et al. 2018), which in accordance with our third hypothesis can further mitigate drought effects on plant productivity due to a higher ability of the soil to retain moisture.

Interestingly, no direct drought effect was observed on microbial C, N and P pools (except for C in HOM soil) at final harvest, but both soil types showed a strong site elevation effect, with opposite directions with respect to soil temperature and moisture. A similar limitation trade-off between soil temperature and moisture has been previously demonstrated for soil respiration by Mills et al. (2014) who showed that, for the same type of soil transplanted along an elevation gradient, moisture was limiting at the warm and dry extreme, while it was temperature at the cold and moist extreme. These contrasting patterns are best illustrated in the RDAs, which showed that the LOM soil was prone to moisture limitation and thus could take advantage of the increased soil moisture at the higher site for increasing microbial biomass. Differently, the HOM soil, which was richer in soil organic matter and could sustain higher moisture, benefited from warmer soil conditions at the lower site in increasing microbial biomass. The microbial biomass C:N, C:P and N:P stoichiometry changed drastically only in the HOM soil, and their increase due to warmer climate was indicative of nutrient limitation for the increased microbial biomass. Warmer conditions did not affect soil extractable N for plant growth and only slightly reduced P-availability in HOM, which in accordance with the fourth hypothesis, indicates that higher soil organic matter content warrants positive effects of warmer and drier climate on microbial activity and growth, rendering these soils less prone to drought stress.

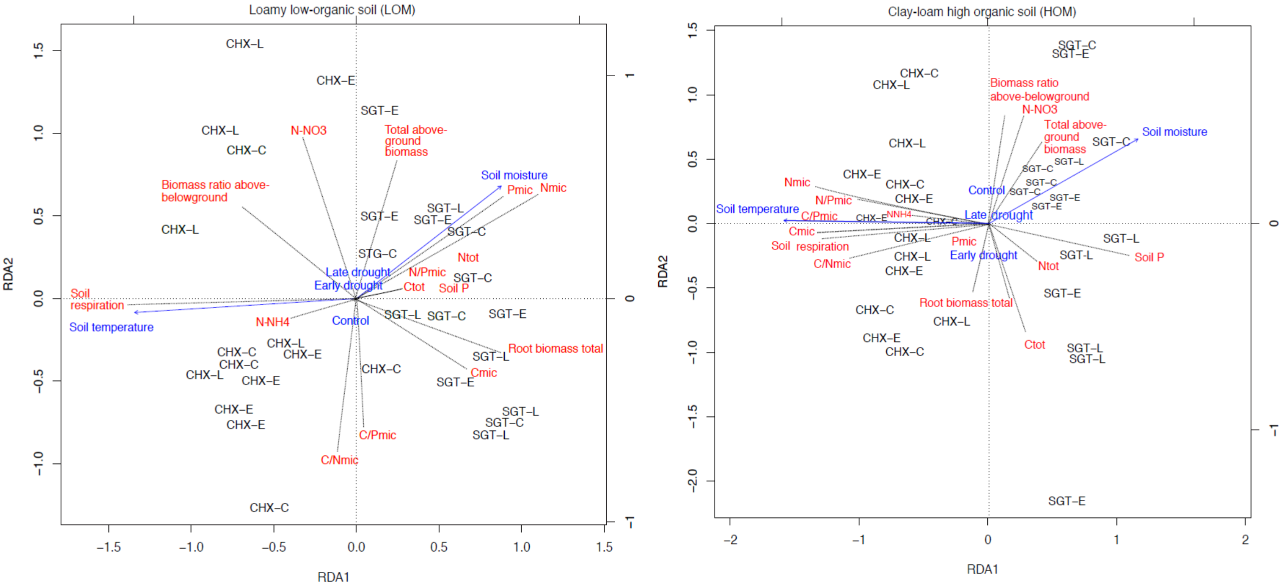


Fig. 5. Redundancy analysis (RDA) of the soil samples (surface 0–12 cm layer of the pot) for the loamy low-organic soil (graph on the left) and the clay-loam highorganic (graph on the right) placed at the low (540m a.s.l) and high (945m a.s.l) sites. Samples are labelled according to the recipient sites, CHX: Chéserex, SGT: St- George and to the treatments, C: control pots, E: early-season drought treatment, L: late-season drought treatment; thus, replicates have the same labels. Response variable are soil biochemical properties and biomass measures. Explanatory variables are treatments (control, early-season and late-season droughts), soil moisture and soil temperature. Left graph: axis 1: 21% (P < 0.001); axis 2: 7% (P=0.10), overall model is significant (P < 0.001);

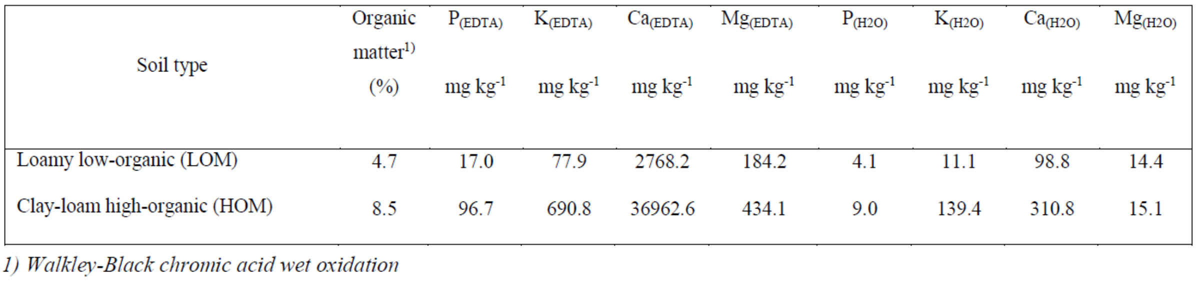
soil temperature and soil moisture are significant ($P < 0.001$). Right graph: axis 1: 30% ($P < 0.001$); axis 2: 4.4% ($P=0.44$), overall model is significant ($P < 0.001$); soil temperature and soil moisture are significant ($P < 0.001$).

Conclusion

In conclusion, our study shows that soils with different physicochemical characteristics respond differently to drought in relation to site climatic conditions and that this impacts on biogeochemistry and plant productivity. Overall, soil with high organic matter content was less affected by drought than soil with low organic matter content. This indicates that soil type and management practices, such as SOM accumulation and organic residue incorporation (e.g. solid manure and/or slurry in pastures, or compost in cropland), represent a promising way to both curtail greenhouse gas emissions and mitigate the effect of increased temperature and drought on plant growth.

9.4.6 Appendix A. Supplementary information

Table S1. Soil Characteristics



Soil type	Organic matter[1)] (%)	$P_{(EDTA)}$ mg kg^{-1}	$K_{(EDTA)}$ mg kg^{-1}	$Ca_{(EDTA)}$ mg kg^{-1}	$Mg_{(EDTA)}$ mg kg^{-1}	$P_{(H2O)}$ mg kg^{-1}	$K_{(H2O)}$ mg kg^{-1}	$Ca_{(H2O)}$ mg kg^{-1}	$Mg_{(H2O)}$ mg kg^{-1}
Loamy low-organic (LOM)	4.7	17.0	77.9	2768.2	184.2	4.1	11.1	98.8	14.4
Clay-loam high-organic (HOM)	8.5	96.7	690.8	36962.6	434.1	9.0	139.4	310.8	15.1

1) Walkley-Black chromic acid wet oxidation

9.4.7 References

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9.5 Appendix 5: Curriculum Vitae

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STRENGTHS

- Knowledgeable in soil carbon dynamics and sequestration with broad field experience and analytical skills in the laboratory
- Skilled at managing, analyzing and presenting data in both verbal and written form
- Proven leaderships, supervisory and time management abilities, developed through six years of work experience directing farming operations in various Latin American countries
- Proactive in proposing alternative solutions

EDUCATION

2016 – present	**Swiss Federal Institute of Technology (EPFL), Lausanne, Switzerland** PhD in Environmental Science and Engineering with focus on Soil Biogeochemistry.
2013 – 2015	**Iowa State University, Ames, Iowa, USA** Master of Science in Crop Production and Physiology
2004 – 2007	**Escuela Agrícola Panamericana - Zamorano, Honduras, C.A.** BSc. in Agronomy with focus in Food Science

LANGUAGES

- Spanish, native speaker
- English, fluent
- Portuguese, intermediate
- French, beginner
- Swiss German, keen to learn

CORE EXPERIENCE

2016 – present

Swiss Federal Institute of Technology (EPFL) – PhD Assistant

Thesis: Alternative land use change scenarios for the expansion of a more sustainable agriculture in the tropics.

Tasks and responsibilities: I am characterizing changes in soil biogeochemical processes and properties in deforestation-free land use change scenarios for the expansion of agriculture. I am doing my PhD thesis in the frame of the SNF-funded multidisciplinary Oil Palm Adaptive Landscapes (OPAL) project. More specifically, I have investigated the effects of pastures and savanna conversion into oil palm plantations on ecosystem carbon pools with a focus on the potential carbon sequestration capacity of soils. I collected samples and data in the field, carried out a broad variety of laboratory analyses to characterize soils chemically and biologically and managed laboratory incubations and soil carbon fractionation procedures. My results suggest that alternative land use change options for the expansion of agriculture in the tropics can reduce the negative impacts of agricultural expansion on important ecosystem services like nutrient cycling, carbon storage and food production. Potential soil's sink carbon capacity and functioning can be further enhanced if better agricultural management practices like use of cover crops and addition of organic amendments are adopted at large scales. With my PhD work, I have developed a strong theoretical foundation in the disciplines of soil's carbon and nutrients biogeochemistry. I also reinforced my analytical skills in the laboratory and on statistical analysis.

2013 – 2015

Iowa State University – Graduate Research Assistant

Thesis: "Amino acid biosynthesis byproducts are a suitable source of nitrogen for corn production"

Tasks and responsibilities: Research focused on determining the suitability of replacing common nitrogen fertilizers with amino acids biosynthesis byproducts for corn production. Growth, developmental and physiological parameters monitored included leaf area index, normalized difference vegetation index, chlorophyll readings (SPAD) and crop phenology. Various productive, quality parameters and nitrogen indexes such as harvest index, nitrogen harvest index, total nitrogen uptake and others were measured and calculated. Our results suggest that amino acid biosynthesis byproducts are a suitable source of nitrogen for corn production and can replace common nitrogen fertilizers without affecting crop productivity and development.

2010 – 2012

Agropiñón/ La Fabril, Manta, Ecuador – Chief of Farm Operations

Tasks and responsibilities:

- Directed teams of agronomists and administrators at both a cotton farm and a Jatropha farm totaling about 1,100 hectares.
- Coordinated and planned all farm activities in the areas of agronomy and administration.
- Prepared and established the annual budget for both the cotton and

the *Jatropha* farm.
- Oversaw all the farm expenses such as general purchases, general service payments, and salaries.
- Analyzed the expenses and income of the company on a monthly basis in order to make decisions affecting profitability.
- Led the research activities regarding the agronomy management to improve *Jatropha* productivity. Performed numerous and distinctive trials regarding agronomic issues.
- Assisted in the agronomic research conducted to evaluate the profitability of introducing commodity crops in marginal lands. Tropical crops included: achiote (*Bixa Orellana*), plantain (*musa spp.*), cacao (*Theobroma cacao*), oil palm (*Elaeis guineensis*), and balsa (*Ochroma pyramidale*)

2010

BEI International, South Haven, Michigan, USA – Manager Assistant

Tasks and responsibilities:
- Participated and assisted with the sales activities of Jatropha harvesters.
- Represented the company at international events to promote sales of Jatropha harvesters.
- Contacted potential customers interested in purchasing mechanical harvesters and evaluated equipment needs.
- Helped with the translations of promotional and sales documents in order to expand retail sales.

2008 – 2009

Agroipsa, Choluteca, Honduras – Research Assistant

Tasks and responsibilities:
- Conducted agronomic research activities examining the Jatropha crop in order to improve its viability both technically and economically. Activities included: fertilization trials, effects of hormonal spraying on fruit and seed productivity, pruning effects, soil preparation, propagation techniques, weed control and uses of co-products.
- Reported the technical results derived from the field studies conducted on a weekly basis.
- Directed, coordinated and supervised the research studies of eight senior undergraduate students from two different Agriculture Colleges located in Honduras.
- Led and supervised all the activities of the greenhouse area where the Jatropha seedlings were produced.
- Managed the collaboration that exists with educational institutions in order to attract senior students eager to conduct thesis research at the farm facilities.

ADDITIONAL EXPERIENCE AND EDUCATION

2019

Institute of Climate-Smart Agriculture, Thunen Institute, Braunschweig, Germany – Research Intern

Tasks and responsibilities:
- Conducted physical and chemical fractionation of soil organic carbon

on samples collected in tropical savannas, pastures and oil palm plantations in Colombia under the direction of Dr. C. Poeplau and A. Don.

2017-2019 **Swiss Federal Institute of Technology (EPFL)** – Teaching Assistant

Tasks and responsibilities:

- Helped with teaching of the master's level class: Multivariate Statistical Methods in Ecology and Environmental Engineering (Prof. A. Buttler)
- Helped with teaching of the master's level class: Experimental Design and Data Analysis with R (Prof. R. Schlaepfer)

2019 **The Graduate Schools for Production Ecology & Resource Conservation (PE&RC) and the Wageningen Institute for Environment and Climate Research (WIMEK)** – Graduate Student, Soil Ecology Course

Tasks and responsibilities:

- Trained on the multifunctional potential of soils

2017 **International Center for Tropical Agriculture (CIAT), Cali, Colombia** – Research Intern

Tasks and responsibilities:

- Soil incubation was conducted to investigate the novel soil-plant trait Biological Nitrification Inhibition (BNI) within the group of Tropical Forages under the supervision of Dr. Jacobo Arango and Idupulapati Rao.

2015 **Organization for Tropical Studies, San José, Costa Rica** – Graduate Student, Winter Course

Tasks and responsibilities:

- Conducted research during a three-weeks graduate course across different tropical ecology research stations in Costa Rica.

2014 **Purdue University, Indiana, USA** – Food Security fellow, Summer Course

Tasks and responsibilities:

- Learned about issues surrounding global food security during the two-week course U.S. Borlaug Summer Institute,.

2008 **National Literacy programme: "Educatodos", Choluteca, Honduras** – Volunteer Literacy Tutor

Tasks and responsibilities:

- Tutored an adult literacy program of the government of Honduras.

2007 **Embrapa, Rio de Janeiro, Brazil** – Research Intern

Tasks and responsibilities:

- Helped with data collection of research projects evaluating the effects of different levels of viscosity of three types of flour on the resulting product of extrusion.

- Participated and cooperated in the development of research theses of M.S. students evaluating the effects of adding water to different flours viscosity on the resulting product of food extrusion.
- Collected data from diverse research projects conducted in the department as part of the daily routine of research working in related studies.

2007

Green Technology Laboratories, Rio de Janeiro Federal University, Brazil – Research Intern

Tasks and responsibilities:

- Participated in the development of research studies focused on the improvement of methodology to obtain biodiesel from alternatives sources such as oils with high acidity and non-conventional oils such as Jatropha.
- Developed laboratory skills to produce biodiesel using different oil and fat sources.
- Gained knowledge evaluating biodiesel quality and fuel characteristics.

2006

Escuela Agrícola Panamericana, Valle del Yeguare, Honduras – Project Assistant

Tasks and responsibilities:

- Served as assistant for the herbarium Paul C. Standley.
- Yeguare initiative. Evaluation of the stage of primary necessities in Liquidambos village in order to analyze the likelihood of granting funds to improve their living conditions.
- Yeguare initiative. Installation of a drip irrigation system in Coyolito Village seeking to help farmers to improve tomato productivity.

2006

Sugar Cane Research Institute, La Troncal, Ecuador – Research Intern

Tasks and responsibilities:

- Participated in research activities focused on the development of new sugar cane varieties.
- Helped with data collection of agronomic research projects such as pest control and plant breeding.

2006

Talento y Efectividad, Tegucigalpa, Honduras – Office Assistant

Tasks and responsibilities:

- Collaborated on office tasks such as preparing PowerPoint presentations, organizing files and typing data collected from interviews or surveys regarding human resources management aspects from different companies.

MENTORING

- Escuela Agrícola Panamericana, Zamorano: co-advisor for 5 BSc. Students
- National Agriculture University of Honduras: co-advisor for 3 BSc. students

MISCELANEOUS

Driving license	Swiss B
IT Skills	R statistical program, SAS statistical software, Adobe Illustrator, Microsoft Office
Peer Reviewer	Plant and Soil
Civil Status	Divorced
Technical skills	Broad variety of laboratory analyses to characterize soils chemically, biologically and physically. Laboratory incubations and soil carbon fractionation procedures. Carbon and nitrogen stable isotopes (^{13}C and ^{15}N). Extensive research and practical agronomical experience in various countries across America and Switzerland.

AWARDS

2018	PhD Mobility Award – Doctoral Program in Civil and Environmental Engineering (EDCE), Swiss Federal Institute of Technology (EPFL). This grant allowed me to make a five-week research visit to the Institute of Climate-Smart Agriculture at the Thunen, Institute in Braunschweig, Germany
2018	Travel Grant – International Union of Soil Sciences (IUSS), Vienna, Austria With this I was able to participate in the 21st World Congress of Soil Science in Rio de Janeiro, Brazil
2015	Student poster competition – ASA-CSSA-SSSA International Annual Meeting, SSSA Division: Soil Fertility & Plant Nutrition, Minneapolis, MN (35 participants), USA First place in M.Sc. student poster competition at the ASA-CSSA-SSSA International Annual Meeting, SSSA Division: Soil Fertility & Plant Nutrition.
2015	Travel Grant – College of Agriculture and Life Science Dean's office Scholarship, Iowa State University, Ames, Iowa The grant covered partially the travel expenses to attend the Tropical Ecology course of the Organization for Tropical Studies (OTS) in Costa Rica, Jan. 2015

2015	Travel Grant – Department of Agronomy, Iowa State University, Ames, USA The grant covered partially the travel expenses to attend the Tropical Ecology course of the Organization for Tropical Studies (OTS) in Costa Rica, Jan. 2015.
2014	Travel Grant – Monsanto Company, Saint Louis, Missouri, USA The grant covered the travel expenses to visit Monsanto Headquarters in St. Louis, Missouri, USA.
2014	Student Fellowship – Purdue University, Indiana, USA The fellowship covered all the expenses related to the participation in the 2-week long learning program on Global Food Security of the U.S. Borlaug Summer Institute.
2013	Student poster competition – 43rd North Central Extension-Industry Soil Fertility Conference, Iowa, USA Third place in M.Sc. student poster competition 43rd North Central Extension-Industry Soil Fertility Conference (28 participants).
2013	Research Assistantship – Iowa State University, Iowa, USA Full graduate research assistantship to pursue M.Sc. program at the Agronomy Department of Iowa State University
2004	Scholarship – Escuela Agrícola Panamericana, Zamorano, Honduras Partial scholarship to pursue B.Sc. program at Zamorano University

PUBLICATIONS

- **Quezada, J.**; Etter, A.; Ghazoul, J.; Buttler, A.; Guillaume, T., 2019: Carbon neutral expansion of oil palm plantations in the Neotropics. **Science Advances**. Vol. 5, no. 11, eaaw4418. doi: 10.1126/sciadv.aaw4418
- Rüegg, J.; **Quezada, J.**; Santonja, M.; Ghazoul, J.; Kuzyakov, Y.; Buttler, A.; Guillaume, T., 2019: Drivers of soil carbon stabilization in oil palm plantations. **Land Degradation & Development**. 30: 1904– 1915. doi : 10.1002/ldr.3380
- Buttler, A.; Mariotte, P.; Meisser, M.; Guillaume, T.; Signarbieux, C.; Vitra, A.; **Quezada, J.**; Bragazza, L.; Gavazov, K., 2019: Drought-induced decline of productivity in the dominant grassland species Lolium perenne L. depends on soil type and prevailing climatic conditions. **Soil Biology and Biochemistry**, 132: 47-57. doi: 10.1016/j.soilbio.2019.01.026
- **Quezada, J**.; Lenssen, A.; Moore, K.; Sawyer, J.; Summer, P., 2015: Amino acid biosynthesis byproducts are a suitable source of nitrogen for corn production. **Field Crops Research**. 184: 123 – 132. doi: 10.1016/j.fcr.2015.09.014

CONFERENCE CONTRIBUTIONS | *talk, °poster

- *T. Guillaume, **J.C. Quezada** and A. Buttler. 2019. Disentangling the effect of soil organic C input and mineralization alteration by management on SOC stabilization in oil palm plantations. Presented April 7th-12th at 21st EGU General Assembly, EGU2019. Vienna, Austria.
- °**J.C. Quezada**, T. Guillaume and A. Buttler. 2018. Long term dynamics of soil organic carbon pools in oil palm plantations following land use change: insights from physical fractionation and natural 13C abundance. Presented August 12th at the 21st World Congress of Soil Science. Rio de Janeiro, Brazil.
- °T. Guillaume, **J.C. Quezada**, and A. Buttler. 2018. Management impacts on soil organic C decomposition and stabilization rates in oil palm plantations. Presented April 4th -13th at 20th EGU General Assembly, EGU2018. Vienna, Austria.
- ***J.C. Quezada**, T. Guillaume and A. Buttler. 2018. Long-term impacts of oil palm cultivation on soil carbon dynamics and biogeochemical soil properties following pasture conversion in Colombia. Presented April 12th at 20th EGU General Assembly, EGU2018. Vienna, Austria.
- °**J.C. Quezada**, T. Guillaume and A. Buttler. 2017. Long-term Impacts of Oil Palm Cultivation Following Pasture Conversion on Soil Carbon and Nutrients Dynamics.. Presented September 5th at 6th International Symposium on Soil Organic Matter. Harpenden, UK.
- ***J.C. Quezada**, T. Guillaume, A. Buttler and J. Ruegg. 2017. Impact of savanna conversion to oil palm plantations on C stocks dynamics and soil fertility. Presented April 25th at 19th EGU General Assembly, EGU2017. Vienna, Austria.
- *T. Guillaume, J. Ruegg, **J.C. Quezada** and A. Buttler. 2017. Soil C dynamics under intensive oil palm plantations in poor tropical soils. Presented April 24th at 19th EGU General Assembly, EGU2017. Vienna, Austria.
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2015. Commercial biosynthesis amino acids byproducts are a suitable replacement for synthetic N fertilizers in corn production. Presented November 16th at ASA-CSSA-SSSA International Annual Meeting. Minneapolis, MN.
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2014. Amino acids and biosynthesis byproducts as nitrogen sources for corn production. Presented November 4th at ASA-CSSA-SSSA International Annual Meeting. Long Beach, CA.
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2014. Utilization of biosynthetically produced amino acid byproducts as nitrogen for corn production. Presented July 27th at the 69th Soil Water Conservation Society International Annual Conference
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2014. Utilization of biosynthetically produced amino acid byproducts as nitrogen for corn production. Presented July 22nd at the Iowa NSF EPSCoR.
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2014. Utilization of biosynthetically producedamino acid byproducts as nitrogen for corn production. Presented June 9th at the Borlaug Summer Institute on Global

Food Security.

- ***J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2014. Corn production using amino acid biosynthesis byproducts as nitrogen fertilizer sources. Presented November 3rd at ASA-CSSA-SSSA International Annual Meeting. Long Beach, United states of America.
- °**J.C. Quezada**, A.W. Lenssen and K.J. Moore. 2013. Replacement of Conventional Source N Fertilizer with Tryptophan Byproduct N does not Influence Corn Grain Yield. Presented November 20th at the 43rd North Central Extension-Industry Soil Fertility Conference.
- °Moreno, C. O., **Rivera, J. C. Q**., Carvalho, C. W. P., Ascheri, J. L. R. Efeito da moagem na preparação de amostras amiláceas. XV ENAAL Congresso Latino Americano de Analistas de Alimentos, 2007, Fortaleza.
- °Ascheri, J. L. R., Carvalho, C. W. P., Silva, E. M. M., Mello, R.; Alcanfor, J. D. X. , **Rivera, J. C. Q**., Moreno, C. O. Características físicas e químicas de misturas de farinhas extrusadas de arroz e inhame (Colacasia spp.). XV ENAAL Congresso Latino Americano de Analistas de Alimentos, 2007, Fortaleza.
- °Boekel, S., Ascheri, J.L.R., Maia M.C., Odicio C.O., **Rivera, J.C.Q.**, Fiuza V.M., Madeira, E.M., Efeito da viscosidade de pasta em misturas extrusadas de arroz, fibra de laranja e farinha de soja. In: XV ENAAL Congresso Latino Americano de Analistas de Alimentos, 2007, Fortaleza.