https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

Diachronic assessment of soil organic C and N dynamics under long-term no-till cropping systems in the tropical upland of Cambodia

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Abstract

No-till (NT) cropping systems have been proposed as a potential strategy to combat soil degradation and global warming by storing soil organic carbon (SOC) and nitrogen (N). Yet, there are ongoing debates about the real benefits of NT systems and factors influencing SOC and N accumulation. Assessing the dynamics of SOC and N on the long-term is needed to fill knowledge gaps and provide robust scientific evidence for potential additional SOC storage. We quantified the changes in SOC and N stocks and fractions down to 100 cm depth from three 13-year-old experiments in a tropical red Oxisol in Cambodia, comparing conventional tillage (CT) to NT monocropping and NT crop rotation systems using a diachronic and equivalent soil mass (ESM) approach. The three experiments comprised maize-, soybean-, and cassava-based cropping system trials, hereafter called MaiEx, SoyEx, and CasEx, respectively. Soil samples were collected in 2021, 10 years after the first sampling in 2011, at 7 depths: 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm. Over the 10-year period (2011-2021), significant impacts on SOC stock and its vertical distribution differed among the NT systems and in the three experiments. In MaiEx and CasEx, the soils under all the NT systems significantly ($P > 0.05$) accumulated SOC stock across the soil depths, with the accumulation ranging from 6.97 to 14.71 Mg C ha^{-1} in the whole profile (0–100 cm). In SoyEx, significant increase in SOC stock was limited to the top 0–20 cm under NT monocropping, whereas NT crop rotation systems had significantly accumulating SOC stock from 0 to 80 cm depths. When considering 0–100 cm as a single stratum, the annual SOC cumulative rate in NT systems ranged from 0.86–1.47, 0.65–1.00, and 0.70–1.07 Mg C ha^{-1} yr^{-1} in MaiEx, SoyEx, and CasEx, respectively. In the top 0–10 cm, NT systems significantly increased C concentration in particulate organic matter (POM) by 115%, 118%, in MaiEx and SoyEx, respectively, and by 37% in CasEx although not significantly.

Similarly, at 0–10 cm depth, NT systems significantly enhanced C concentration in the mineral-associated organic matter (MAOM) by 33%, 21%, in MaiEx and SoyEx, respectively. Significant increase of C in MAOM was also observed from 0 to 40 cm in CasEx. In contrast, total N stock in NT systems increased in the surface 0–5 cm depth but decreased below 10 cm and in the whole profile (0–100 cm), particularly under NT monocropping with an annual loss rate of -0.10 and -0.17 Mg N ha^{-1} yr^{-1} in SoyEx and CasEx, respectively. Although NT systems increased N concentration in POM in the top 0–10 cm of MaiEx and SoyEx, a decreasing trend was observed below 10 cm depth. The N concentration in POM under NT systems in CasEx also decreased with soil depth. From 2011 to 2021, N concentration in MAOM under NT systems remained stable in MaiEx and SoyEx in the top 0–5 cm, but significant decreases in MaiEx and CasEx below 5 cm.

Our findings suggest that adopting NT cropping systems with diverse crop and cover crop species and high biomass C inputs in the long-term leads to SOC accumulation not only in the surface but also in deeper layers, by increasing both the C pools in the POM and MAOM size fractions, even on the cassava-based system, which is believed to be an annual crop that could cause serious soil fertility depletion. This study highlights the potential of NT cropping systems to store SOC over time, but raises questions about soil N dynamics.

1 Introduction

Land and soil degradation is a global challenge with consequences not only for food and nutrition security but also for livelihoods, environmental pollution, climate change, water scarcity, and biodiversity. The main processes that cause soil degradation are water and wind erosion, chemical depletion, physical deterioration, decline in soil organic carbon (SOC) pools, loss in biodiversity, acidification, and salinity (Lal, 2015a; Stavi and Lal, 2015; Dragović and Vulević, 2020; Barbier and Di Falco, 2021). It was estimated that about 562 million ha of land are degraded worldwide, with 5 to 10 million ha of land lost each year as a result of severe degradation (Stavi and Lal, 2015; Nkonya et al., 2016). The major factors contributing to soil degradation are deforestation and land clearance, the overuse of agrochemicals, and inappropriate agricultural management practices (Dragović and Vulević, 2020). Tropical soils have the highest risks of degradation due to the combination of high rainfall intensity and the ongoing intensification of agriculture to meet the food demand of a fast-growing population, which is also constrained by the limited availability of land to be converted to cropland (Barbier and Hochard, 2018; Craswell and Lefroy, 2001; Barbier and Di Falco, 2021).

Cambodia, located in the tropical region of Southeast Asia, is one of the highest land degradation hotspots in the world, and about 60% of the country's population reside in these hotspot areas. In the last two decades, human-induced activities including deforestation, land clearance for agriculture, climate change, and inappropriate farming practices have further worsened Cambodia's already poor soil fertility (UNCCD, 2018; Ken et al., 2020; ADB, 2021). Over the past two decades, 30%, or about 4.24 million ha, of forest areas were converted to croplands, putting pressure on natural resources, biodiversity, and threatening the provision of several ecosystem services (World Bank Group, 2023). In the Northwest rainfed uplands, like in other parts of the country, studies on soil erosion at field scale and modelling reported that the annual soil loss rate in conventional plough-based tillage (CT) ranged from 0.33 to more than 80 Mg soil ha^{-1} yr^{-1}, depending on soil type and land slope (CARDI, 2017; Nut et al., 2021; Sourn et al., 2022). The amplitude of soil erosion increased by 41% from an annual erosion rate of 2.92 Mg soil ha^{-1} yr^{-1} in 1998 at the beginning of the forest conversion to agriculture with extensive, more diversified farming practices to 4.98 Mg soil ha^{-1} yr^{-1} in 2018 under CT maize and cassava-based monocropping systems (Nut et al., 2021; Sourn et al., 2022, 2023). It was estimated that approximately 3–4 mm of topsoil is washed away annually for this Northwestern region of Cambodia (Nut et al., 2021; Sourn et al., 2023). Erosion induces soil degradation and SOC loss (Polyakov and Lal, 2004). It was estimated that from 2000 to 2010, Cambodia lost approximately 1.98 million Mg C in the top 0–30 cm depth as the consequence of forest conversion to other land uses (MAFF, 2018).

SOC serves as the foundation of soil physical, chemical, and biological processes that sustain essential ecosystem functions, and it is the reservoir of plant nutrients and energy for biota. Therefore, adopting sustainable management practices that lead to increase in SOC content (Beillouin et al., 2023) is part of the key strategies to reverse the soil degradation trends and to minimize the economics and environmental impacts related to land degradation (Lal, 2015a; Obalum et al., 2017). Studies reported that agricultural practices, in particularly those based on CT, weaken soil structure, accelerate soil erosion, and deplete SOC stock (Tivet et al., 2013; Sá et al., 2014; Briedis et al., 2018; Oliveira et al., 2020; Tiecher et al., 2020). By contrast, conservation agriculture (CA), defined by three key principles, (i) minimum or no soil disturbance, (ii) permanent soil cover with mulch or cover crops, and (iii) crop diversification through rotation or association, is a potential strategy to overcome soil degradation (Luo et al., 2010; Lal, 2015b; Powlson et al., 2016; Obalum et al., 2017). No-till cropping systems (NT), are part of the conservation agriculture practice approach, and involve a range of practices with a reduction or an absence in soil tillage and a high diversity of plant species, amount and frequency of biomass-C inputs (e.g., main crops and cover crops). The benefits of CA and NT cropping systems on soil health improvement have been reported worldwide. Diversified NT cropping systems enhance the SOC stock in the topsoil layer only after a several years of implementation (Hok et al., 2015) and pools (Sá et al., 2014; Briedis et al., 2018; Sithole et al., 2019; Cooper et al., 2021; Rodrigues et al., 2022), especially through an increase in physical protection of SOC inside soil aggregates (Six et al., 2002; Sithole et al., 2019; Rodrigues et al., 2022). In addition, numerous studies have reported the co-benefits of NT cropping systems on soil health enhancement (Pheap et al., 2019; Koun et al., 2023), increased water infiltration, reduced soil erosion (TerAvest et al., 2015; Sithole et al., 2019), and enhanced microbial activities (Hok et al., 2018) and abundance (Lienhard et al., 2013). Yet, there are still arguments about the benefits of CA and NT cropping systems and associated factors that determine SOC accumulation. In addition, documentation of SOC fractions is desirable for a better understanding of SOC dynamics and stabilization processes (Lavallee et al., 2020). In a meta-analysis with the majority of the studies collecting samples between 0.15 and 0.3 m depth, Powlson et al., 2016 reported that SOC accumulation rate under CA systems ranged from 0.16 to 0.49 Mg C ha^{-1} yr^{-1} in tropical soils in the Indo-Gangetic Plains and from 0.28 to 0.96 Mg C ha^{-1} yr^{-1} in Sub-Saharan Africa. In a Ferralsol in Zimbabwe, Shumba et al., (2024) reported a SOC accumulation rate of 0.13 Mg C ha^{-1} yr^{-1} in the 0–5 cm layer only. However, in meta-analyses, Angers and Eriksen-Hamel (2008) and Luo et al., (2010) found that conversion from CT to NT only changed the SOC distribution in the soil profile but did not significantly increase SOC stock in the whole profile. Boddey et al., (2010) and Xiao et al., (2020) reported that NT significantly increased SOC stock only at the soil surface but not the deeper layers. It is therefore crucial to quantify SOC change in subsoil when assessing the impact of practices, especially in CA and NT systems. The amount, quality and frequency of the crop residues added to soil under a range of climate-driven decomposition rates, soil mineralogy and profile characteristics are important factors to consider to increase SOC stocks (Paustian et al., 1997; Six et al., 2002; Bayer et al., 2006; Ogle et al., 2012; Virto et al., 2012). It has been suggested that the amount of biomass-C inputs was the main factor explaining the variability in SOC storage between sites under NT (Virto et al., 2012). In a synthesis from tropical soils, Fujisaki et al., (2018) reported that the amount of biomass-C inputs was the main factor driving a positive C budget, enhancing C and N transformations, flow, and SOC stock change. In a meta-analysis in Sub-Saharan Africa, Corbeels et al., (2019) found that not disturbing the soil alone does not lead to an increase in SOC stock, but CA systems combining the three principles could. It therefore seems that there is a hierarchy in CA principles to increase SOC stock, the most important one being the permanent soil cover, followed by a reduction in soil tillage and improved rotations (Shumba et al., 2024). This has been confirmed in a recent second-order meta-analysis where crop residue retention and cover crops were the most efficient CA practices to increase SOC (Beillouin et al., 2023). In addition, using improper methods could mislead the assessment of the long-term impact of management practices on the SOC stock. Neto et al., (2010) and Junior et al., (2013) revealed that the synchronic approach led to overestimated SOC accumulation from long-term experiments in Brazil due to spatial heterogeneity and initial land use history. They highlighted that diachronic soil sampling should be used for assessing soil SOC storage rates due to changes in land-use or management patterns. A change in soil bulk density is often observed when comparing CA and NT systems to CT, due to differences in tillage but also to root systems of cover crops. It is therefore required to estimate SOC change using an equivalent soil mass approach instead of a fixed depth approach (Ellert and Bettany, 1995).

Cambodian soils are seriously threatened by inappropriate agricultural systems. The returns on taking actions against land degradation are estimated at 3 US dollars for every dollar invested in restoring degraded land in Cambodia. Therefore, taking actions to reverse the trend of soil degradation through restoration and adopting sustainable agricultural management practices highlights the strong economic benefits while combating soil degradation in the country (UNCCD, 2018). Since 2004, the conservation agriculture research for development programs have been initiated by the joint-collaboration between the General Directorate of Agriculture (GDA) and Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France. CA and NT cropping systems have been promoted to smallholders in various agroecosystems in the country since 2009. The early effects of NT cropping systems on soil health, and SOC sequestration have been reported in several studies (Hok et al., 2015, 2018, 2021; Pheap et al., 2019; Suong et al., 2019; Sar, 2021; Koun et al., 2023), however, the information on the impact of long-term NT systems on the changes in SOC stock remains scarce in the country as well as in Southeast Asia. There is a need to document the long-term changes in SOC stocks under CA and NT cropping systems to fill in the knowledge gaps as well as provide robust evidences to land use planners and policymakers. This could be profitable not only for Cambodia but also for the whole region.

Therefore, this study aimed to quantify the impacts of CT and different NT cropping systems on the changes in SOC and total nitrogen (N) stocks and fractions over time (2011–2021) in Cambodia’s tropical red Oxisol using diachronic and equivalent soil mass (ESM) approaches. We hypothesized that implementation of the three core technical principles of CA would significantly enhance the SOC stocks including in the subsoils. In addition, calculating SOC stock using the diachronic approach would prevent a biassed estimation of the SOC accumulation when compared to the synchronic approach.

2 Materials and Methods

2.1 Study site description

The study was conducted at Bos Khnor Conservation Agriculture Research Station, the oldest CA research station in Southeast Asia, which belongs to the General Directorate of Agriculture (GDA), Department of Agricultural Land Resources Management (DALRM). It is located in Chamkar Leu district, Kampong Cham province (12°12'31.0"N 105°19'07.0"E, 118 m above sea level). The details of the study site were reported in Hok et al., (2015). Briefly, the soil of the study site is classified as a red Oxisol (USDA, 1999) or a Ferralsol in the world reference base (WRB) for soil resources (IUSS Working Group WRB, 2015), with 1.3% sand, 29% silt, and 69% clay in the 0–20 cm and gradually increasing with soil depth to 78 % clay at 20–100 cm. The clay fraction is mainly made of kaolinite (Hok et al., 2015). The research site's climate is defined as tropical monsoon, corresponding to the Am Köppen climate classification, with two main seasons: the wet season from May to October and the dry season from November to April. The mean annual temperature from 2009–2021 was 27.5°C, while the average annual minimum and maximum temperatures were 22°C and 35°C, respectively. The annual rainfall during the last 13 years ranged between 1,650 and 2,000 mm.

2.2 Experimental design, treatment description, and crop management

The detailed history of the research site, experimental design, treatment description, and fertilizer application were reported in Hok et al., (2015) and Pheap et al., (2019). Our study covers three experiments, implemented in 2009, including (i) maize (*Zea mays* L.) (which was a former rice (*Oryza sativa* L.)-based trial from 2009 to 2019 and shifted to maize-based trial in 2020), (ii) soybean (*Glycine max* L.)-, and (iii) cassava (*Manihot esculenta* Crantz)-based cropping system trials, hereafter called

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

MaiEx, SoyEx, and CasEx, respectively. These represent the most important annual upland crops in Cambodia as well as in some Southeast Asian countries. The experiments are arranged in a randomized complete block design with three replicates. The elementary plot dimensions are 8m x 37.5m, equivalent to 300 m^2. Each experiment consists of four (4) treatments: (i) conventional tillage (CT) in which the main crops, i.e., maize (Mz), soybean (Sb), and cassava (Cs) are mono-cropped with land preparation done by disc plowing (CT-Mz, CT-Sb, and CT-Cs); (ii) no-tillage mulch-based cropping systems (NT), in which the main crops (maize, soybean, and cassava) are cropped in a one-year frequency pattern under CA management (NT1-Mz, NT1-Sb, and NT1-Cs) along with addition of cover crops; (iii) and (iv) NT systems in which the main crops (i.e., soybean and cassava), were grown in a bi-annual rotation with maize in the case of SoyEx (NT2-Sb, NT3-Sb) and CasEx (NT2-Cs, NT3-Cs), respectively, while the maize was grown in a bi-annual rotation with soybean in the case of MaiEx (NT2-Mz, NT3-Mz) under CA management along with addition of cover crops.

For main crop residue management in MaiEx and SoyEx, all crop residues were retained in the soil in all the tillage systems. In CasEx, all the cassava fallen leaves and branches were retained in the soil, while 100% of the cassava main stems and original cuttings were completely removed from the plot after harvest under CT-Cs, representing standard farmers' practices. For all the NT-Cs, all the cassava fallen leaves and branches were returned to the soil, while 50% of the cassava main stems and 100% of the original cuttings were retained in the soil and then crimped to speed up the decomposition process and facilitate field operation implementations in the following cropping season. The residues of all the cover crops were left as mulch under all the NT systems in all the experiments.

There were a few adjustments on the cropping systems over the experimental period from 2009 to 2021, especially with the use of cover crops, crop varieties, and mineral fertilizer application types and rates. Details of main crops and cover crop successions and the cumulative amount of the aboveground biomass-C and N inputs from the crops are presented in Table 1. The C inputs were estimated from the dry aboveground biomass inputs recorded prior to the termination of the cover crops and at grains/tuber harvest for the main crops. The C and N inputs from the root systems were not recorded nor even estimated based on literature. In the case of missing data of aboveground biomass, the amount of biomass was estimated using the average of recorded data over time as reference in the case of cover crops and grain and/or tuber-aboveground biomass ratio for the main crops (e.g. rice, maize, soybean and cassava). In addition, the cumulative and annual N inputs were estimated from the amount of the cumulative and annual C inputs, respectively, by applying available C/N ratio values of each plant species that were yielded from the C and N concentration analysis by dry combustion.

For land preparation, the CT plot is ploughed twice to 15-20 cm depth using a 7-disc plough after early rains at the beginning of the wet season and then before the main crop cultivation. If sufficient early rain falls were received at the beginning of the wet season (in the 3rd week of March), Sesame (*Sesamum indicum* L.) and mung bean (*Vigna radiata* (L.) R. Wilczek) were sown manually under CT treatment in SoyEx and MaiEx, respectively, as early-cycle cash crops (April to June) prior to the main crops, i.e., soybean or maize (from July to November). If that was not the case, the CT plots remained fallow with the growth of natural grasses and broad leaves until the main cycle crops. These cropping systems represent the standard farmers' practices. Under the NT systems (NT1, NT2 and NT3), a long cycle cover crop i.e., stylo (*Stylosanthes guianensis* (Aubl.) Sw.) was used as a cover crop and grown in association with the main crops. This cover crop was sown in the middle of the inter-row at 0, 15, 35 days after the sowing of the main crops, i.e., maize, cassava, rice, respectively, and by seed broadcasting at the first yellow leaves of soybean, approximately 4 weeks before harvest. In addition, if the development and/or density of the cover crop sown the previous year was considered insufficient, pearl millet (*Pennisetum typhoides* (L.) Morrone) or sorghum (*Sorghum bicolor* (L.) Moench) was sown alone for the treatments planted with soybean or mixed with sunhemp (*Crotalaria juncea* L.) and cowpea (*Vigna unguiculata* (L.) Walp.) for the treatments planted with maize at the beginning of the rainy season as short-cycle cover crops. The cover crops were then grown for 60–75 days prior to the main cycle of rice, maize, or soybean. The main crops (rice, maize and soybean), both under CT and NT management, as well as the cover crops (at the beginning of the rainy season) were sown by a NT planter (Fitarelli pulled by power tiller, Vence Tudo, or Seamato lifted or pulled by tractor). From 2009 to 2020, cassava was planted along the furrows drawn by chiselling at 0.8 m spacing to approximately 20 cm depth, and then it was planted by a NT cassava planter (Planticenter) in 2021. Under the NT systems, the cover crops were terminated by crimping followed by the application of a mix of non-selective herbicides, i.e., glyphosate [N (phosphonomethyl) glycine] and 2,4-D [2,4-dichloro-phenoxyacetic acid], at a rate of 960 and 720 g active ingredient (a.i) ha^{-1}, respectively.

Since 2009, soil amendment was done with thermo phosphate (16% P_2O_5, 31% CaO and 16% MgO) at the end of dry season (early April), and then basal fertilizers and top dressings on the main crops were applied with different rates of N, P, K depending on the types and phenological stage of each main crops using diammonium phosphate (18% N, 46% P), ammonium sulphate (16% N, 20% P), potassium chloride

(60% K), and urea (46% N). The application of the fertilizer inputs to each main crop are detailed in Table 2.

Table 1. Experiments, cropping systems and crop sequences, and associated cumulative and annual aboveground C and N inputs during the experimental period (2009-2021).

Experiments and cropping systems[a]	Crop sequences from 2009 to 2021[b]	C input (Mg ha^{-1})		N input (Mg ha^{-1})	
		Cumulative	**Annual**	**Cumulative**	**Annual**
MaiEx					
CT-Mz	R – Mu/R – Mu/R – Mu/R – Mu/R – R – R – R – R – R – R – Mz – Mz	28.60	2.20	0.64	0.05
NT1-Mz	Mi/R – Mi/R – Mi/R – St/R – St/R – St/R – St/R – St/R – St/R – St/R – St/R+St – St/R+St – Mi+Su+Co/M – Mi+Su+Co/M	67.70	5.21	1.50	0.12
NT2-Mz	Mi/R – Mi+Su+St/Mz – Mi+Su+St/R – St/Mz+St – St/R+St – St/Mz+St – St/R+St – St/M+St – St/R+St – St/Mz+St – St/So+Su+R – Mi+Su+Co/Mz – So+Su+Co/Sb	73.08	5.62	1.62	0.12
NT3-Mz	Mi/Mz – Mi+P+St/R – Mi+Su+St/Mz+St – St/R+St – St/Mz+St – St/R+St – St/Mz+St – St/R+St – St/Mz+St – So+Su/R – St/Mz+St – So+Su+Co/Sb – Mi+Su+Co/Mz	70.12	5.39	1.56	0.12
SoyEx					
CT-Sb	Sb – Sb – Se/Sb – Sb – Se/Sb – Sb – Sb – Sb – Rb – Sb – Sb – Sb – Sb	23.18	1.78	0.52	0.04
NT1-Sb	Mi/Sb+Br – Mi/Sb+Br – Mi/Sb+Br – Mi/Sb+St – St/Sb+St – St/Sb+St – St/Sb+St – St/Sb+St – So+St/Rb – Rb/So+Sb – So+Su/Sb – So+Su+Co/Sb – So+Su+Co/Sb	65.09	5.01	1.45	0.11
NT2-Sb	Mi/Sb+St – Mi+St/Mz+Br – Mi/Sb+St – Mi+Su/Mz – So/Sb+St – So+Su/Mz – So+St/Sb – So+Su/Mz+St – St/Rb – Rb+So/Mz – So+Su/Sb – Mi+Su+Co/Mz – So+Su/Sb	71.13	5.47	1.58	0.12
NT3-Sb	Mi/Mz+Br – M/Sb+St – Mi+Su/Mz+St – St/Sb+St – So+Su/Mz – So+Su/Sb – So+Su/Mz – So+Su/Sb – So+Su/Mz – So+Su/Sb – So+Su/Mz – So+Su+Co/Sb – Mi+Su+Co/Mz	78.94	6.07	1.75	0.13
CasEx					
CT-Cs	Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs – Cs	17.64	1.36	0.39	0.03
NT1-Cs	Cs+St – St/Cs+St – St/Cs+St – St/Cs+St – Cs+St – Cs+St – Cs+St – Cs – Cs – Cs – Cs – Cs – Cs	46.92	3.61	1.04	0.08
NT2-Cs	Cs+St – Mi+Mz+St – St/Cs+St – Mi+Su/Mz+St – St/Cs+St – St/Mz+St – St/Cs – St/Mz+St – St/Cs – So+Su/Mz – So+Su/Cs – Mi+Su+Co/Mz – Cs	64.25	4.94	1.43	0.11
NT3-Cs	Mi/Mz+St – St/Cs+St – Mi+Su/Mz+St – St/Cs+St – Mi+Su/Mz+St – St/Cs+St – St/Mz+St – St/Cs – So+Su/Mz – So+Su/Cs – So+Su/Mz – Cs – Mi+Su+Co/Mz	67.10	5.16	1.49	0.11

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

[a] MaiEx: maize-based cropping system trial; SoyEx: soybean-based cropping system trial; and CasEx: cassava-based cropping system trial; CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till mulch-based cropping systems associated with different crop sequences.

[b] Br: brachiaria (*Brachiaria ruziziensis* R.Germ. & C.M.Evrard); Co: cowpea (*Vigna unguiculata* (L.) Walp.); Cs: cassava (*Manihot esculenta* Crantz); Mi: millet (*Pennisetum glaucum* (L.) R.Br.); Mu: Mungbean (*Vigna radiata* (L.) R. Wilczek) Mz: maize (*Zea mays* L.); P: pigeon pea (*Cajanus cajan* L.); R: rice (*Oryza sativa* L.); Rb: ricebean (*Vigna umbellata* (Thunb.) Ohwi & H. Ohashi); Sb: soybean (*Glycine max* L.); Se: sesame (*Sesame indicum* L.); So: Sorghum (*Sorghum bicolor* (L.) Moench); St: stylo (*Stylosanthes quianensis* (Aubl.) Sw.); Su: sunnhemp (*Crotalaria juncea* L.); "–" indicates the period between the year; "/" indicates relay cropping with varying planting dates; and "+" indicates crops planted in association (same or staggered sowing dates). The C inputs were estimated from the amount of aboveground biomass of each crop; the belowground biomass was not included.

Table 2. Mineral fertilizer rates applied to the main crops during the experimental period (2009-2021).

Annual mineral fertilizer rates (kg ha^{-1})^a	**Crops**	**Year**													**Total fertilizer input**
		2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**	**2016**	**2017**	**2018**	**2019**	**2020**	**2021**	
N in $CO(NH_2)_2$	Cassava	92	69	69	69	69	69	69	69	69	69	69	69	69	920
	Maize	92	69	69	69	69	46	46	46	46	46	46	46	46	736
	Rice	69	46	46	46	46	46	46	46	46	46	46			529
	Soybean	23	23	23	23	23									115
N in $(NH_4)_2SO_4$	Maize						24	24	24	24	24	24	24	24	192
N in $(NH_4)_2HPO_4$	Soybean						18	18	18	18	18	18	18	18	144
P_2O_5	All crops	80	32	32	32	32	32	32	32	32	32	32	0	0	400
P in $(NH_4)_2SO_4$	Maize						30	30	30	30	30	30	30	30	240
P in $(NH_4)_2HPO_4$	Soybean						46	46	46	46	46	46	46	46	368
K_2O	Cassava	60	90	60	60	60	60	60	60	60	60	60	60	60	810
	Maize	60	30	30	30	30	30	30	30	30	30	30	30	30	420
	Rice	60	30	30	30	30	30	30	30	30	30	30			360
	Soybean	60	60	60	60	60	60	60	60	60	60	60	60	60	780

$CO(NH_2)_2$: Urea (46.0.0); $(NH_4)_2SO_4$: Ammonium sulphate (16.20.0); $(NH_4)_2HPO_4$: Diammonium phosphate (18-46-0); P_2O_5: Thermo phosphate (0.18.0); and K_2O: Potassium chloride (0.0.60).

2.3 Soil sampling and processing

The study was a diachronic analysis from 2011 to 2021. In 2009, prior to the establishment of the experiments, soil and bulk density (ρb) samples were collected as the pre-experiment (PE) from three randomly selected sampling points per replicate of each experimental location at four depths: 0–5, 5–10, 10–20, and 20–30 cm. The individual soil samples from the same depth and replicate were composited, resulting in three composites per depth and per experiment. The composite samples were oven dried at 40°C and sieved through a 2mm mesh for chemical property analysis. Bulk density samples were collected using core samplers of 5 cm in diameter and 5 cm in height, oven dried at 105°C for 48 h.

In November 2011, soil sampling was conducted to assess the early effects of tillage systems on soil organic C and N concentrations and stocks. The details of the sampling are described in Hok et al., (2015). Briefly, two pits (1m x 1m) were opened per elementary plot for soil and ρb sample collection. Individual samples for chemical analysis were collected from two undisturbed sides of each pit at 0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm. The individual samples from the same depth and the same pit were composited, and as a result, two composite samples per layer were collected per elementary plot. The composites were oven-dried at 40°C before being softly disrupted, sieved through a 2-mm sieve, and homogenized. Soil bulk density samples were taken from the same two undisturbed sides of each pit at the same soil depths as for SOC analysis using core samplers of 5 cm in diameter and 5 cm in height. The soil cores were oven-dried at 105°C for 48 h. Similarly, in 2011, six subplots were delimited for soil sampling in an area of roughly 17 ha in the adjacent reference vegetation (RV), which served as a baseline for comparison with the three cropping systems. The reference vegetation site was located approximately

500 m from the experimental plots. The vegetation composition of RV was an old coffee plantation grown under the shade of *Leucaena glauca* (Lam.) de Wit that was planted in 1990. The crop history here was the same as that of the experimental plots from 1937 to 1990 after the conversion of the natural forest to cropland.

In December 2021, we re-sampled the soil to assess the changes in ρb, SOC and N concentrations and stocks ten years after the study conducted by Hok et al., (2015). The samples were collected at the same 7 layers. From each treatment and replicate, we collected four individual samples by an automatic soil column cylinder auger (Eijkelkamp, the Netherlands) in a diagonal "X" shape from four points within each plot, avoiding overlapping on the pits opened in 2011. In addition, we dug a 1 m x 1 m pit in the middle of each plot for sample collection; three individual soil samples and three ρb cores were collected from three undisturbed sides of the pit at each depth. Soil samples were air-dried at room temperature, gently broken down, and sieved through a 2 mm mesh sieve. Finally, the seven individual samples from the same layer were mixed and homogenized to make a composite sample per elementary plot. The samples of ρb were oven-dried at 105°C for 48 h.

2.4 Soil organic C analyses

The SOC and total N concentration of the soil samples collected in 2009 and 2011 were determined by dry combustion using an elemental CN analyzer (TruSpec CN, LECO, St. Joseph, USA). The details of the analysis were described in Hok et al., (2015). Sub-samples of the composite soils (n = 3 per layer) collected in 2021 were finely ground (<150 µm) before analysis for total C and N by dry combustion using the LECO® CHN628 analyzer at the Sustainable Agroecosystems Lab, ETH Zurich University, Switzerland.

2.5 Soil organic C and N stocks calculation

In this Oxisol, there was no coarse fraction (i.e. gravels) left after sieving at 2 mm. Therefore, the bulk density of soil equals the bulk density of fine earth. To avoid inaccurate stock calculation due to differences in bulk density between treatments when using the fixed depth method, the equivalent soil mass (ESM) approach was applied to compute SOC and N stocks (Ellert and Bettany, 1995; Von Haden et al., 2020; Fowler et al., 2023). Since the ρb of the treatments differed between the two sampling years (2011 and 2021) and the reference vegetation at each sampling depth (Table S1 in the supplementary materials), we defined the reference soil mass as the lowest soil mass observed at each sampling depth, regardless of sampling years, cropping systems or land use. For this reference, soil mass layers (480, 518, 1061, 1873, 1766, 1809, and 1779 Mg ha^{-1}) corresponded to the depth layers (0–5, 5–10, 10–20, 20–40, 40–60, 60–80, and 80–100 cm, respectively). We applied these reference soil masses to compute the SOC and N stocks in 2021 and recalculated the stocks of the PE, RV and the treatments of the three experiments collected in 2011.

To correct for differences in ρb, SOC and N stocks were computed according to Eq. 1 and 2.

$$M_{(Soilmin,i)} = \rho b_{(i)} \times T_{(i)} \times 1000 \qquad (eq.1)$$

$$SOCstock = \sum(i=1)^{\wedge}n\ [(M_{(soilmin,i)} \times conc._{(i)}) + ((M_{(soil,i)} - M_{(soilmin,i)}) \times conc._{(i-1)})] \times 0.001 \text{ (eq. 2)}$$

Where: $M_{(soilmin,i)}$ is the minimal soil mass per unit area in the ith layer (Mg ha^{-1}) recorded over the treatments and used as a reference. $\rho b_{(i)}$ is the bulk density of the ith layer (g cm^{-3}). $T_{(i)}$ is the thickness of the ith layer (m). $conc._{(i)}$ is the concentration of SOC in ith layer. $conc._{(i-1)}$ is the concentration of SOC in i - 1th layer. $M_{(soil,i)}$ is the designated soil mass of each layer (i.e., the maximum soil mass). The numbers 1000 and 0.001 are unit conversion coefficients.

We defined delta stock of SOC and N, as the stock change within the same treatment and depth between 2021 and 2011 sampling years (diachronic) and calculated it as follows:

$$\Delta SOCstock.diach = SOCstock_{treatment(i)2021} - SOCstock_{treatment(i)2011} \qquad (eq.3)$$

Where: i represents the treatments.

To compare the synchronic and diachronic approaches for SOC stock change, the stock change estimated by the synchronic approach was computed as follows using the CT treatment as the control treatment:

$$\Delta SOCstock.synch = SOCstock_{NT(i)2021} - SOCstock_{CT2021} \qquad (eq.4)$$

Where: NT(i) represents NT1, NT2, and NT3 treatments.

The SOC and N stock change (accumulation or loss) rates (Mg C or N ha^{-1} yr^{-1}) of each treatment were calculated by dividing Δ SOC stock by the number of years between the 1st and 2nd samplings (10 years):

$$SOCstockchangerate_{treatment(i)} = \frac{\Delta SOCstock_{treatment(i)}}{10} \qquad (eq.5)$$

2.6 Particle-size fractionation of soil organic matter

The soil organic C was physically fractionated using a sub-sample of the composite soil for all the treatments and seven depths. The particle-size fractionation was implemented in accordance with the procedure described in Hok et al., (2015). Briefly, 40 g of soil samples were dispersed in a solution of 1.25 g of sodium hexametaphosphate and 100 mL of deionized water and stored at 10 °C for 16 hours. The sample was then horizontally shaken at 100 rpm for 8 hours with three 10-mm-diameter agate balls. The soil mixture was wet-sieved with deionized water through a 53-µm sieve to get the proportion of particulate organic matter (POM) sized between 53µm and 2,000µm. The < 53-µm fraction was flocculated with 2-g $CaCl_2$ in a 1-L glass cylinder and left overnight for sedimentation. The supernatant was syphoned after full sedimentation. This < 53-µm fraction is made up of mineral-associated organic matter (MAOM). The two fractions were oven-dried at 40°C until reaching constant weight and finely ground for determining C and N concentrations by dry combustion using the LECO® CHN628 analyzer at the Sustainable Agroecosystems Lab, ETH Zurich University, Switzerland.

2.7 Statistical analysis

Statistical analysis was computed using R software, version 4.3.1 (Core Team, 2023). Linear mixed models (lmerTest package) were fitted on all data: sampling years, soil depths, and treatments were defined as the fixed factors, while the replicates were defined as the random factors. Prior to the analysis, the normality of each variable was checked by Shapiro's test. Then we applied Levene's test to check the homoscedasticity of the data. A diachronic approach was used to assess statistical significance between the two sampling years (i.e., 2021 vs. 2011) of the same treatment at the same soil depth by analysis of variance with Fisher tests (degrees of freedom calculated by Satterthwaite method), computing of estimated marginal means (EMMs) and p-value adjustment using the Tukey method. The same approach was used to compare SOC stocks between treatments in the same sampling year, calculated at equivalent soil mass and using the synchronic approach. In addition, we applied the same statistical procedures to assess the statistical significance of cumulative SOC and N stocks.

3 Results

The effects of cropping systems on the concentrations, stocks of SOC and N as well as their fractions in the physical size classes between 2011 and 2021 varied among the three experiments and across the soil profile.

3.1 Cropping system effects on SOC and N concentrations and stocks

The concentrations of SOC and N in 2011 and 2021 are presented in Fig. 1 and Fig. 2, respectively, with the duplicates provided in Table S2 and S3 in the supplementary materials. Table 3 and 4 show the SOC and N stocks in 2021 and the stock change rate between 2011 and 2021, respectively. The SOC and N stocks in 2011 are provided in Table S4 in the supplementary materials.

3.1.1 Maize-based experiment

The SOC concentration of all the treatments in 2011 and 2021 was highest in the topsoil (0–5 cm) and decreased with soil depth (Fig. 1A and 1B). From 2011 to 2021, SOC concentration under NT-Mz crop rotation systems (average of NT2-Mz and NT3-Mz) significantly increased by 50%, 24%, and 15% at 0–5, 5–10, and 10–20 cm depth, respectively. Significant increase was still observed under NT2-Mz at 20-

40 cm depth. NT1-Mz significantly increased SOC concentration over the whole soil profile by 68%, 21%, 16%, 17%, 23%, and 16% at 0–5, 5–10, 20–40, 40–60, 60–80, and 80–100 cm, respectively (Fig. 1B) when compared between 2021 and 2011. In the case of CT-Mz, except for the significant increase observed in the 10–20 cm layer, the SOC concentration remained neutral at 0–10 and 20–80 cm, then significantly decreased at 80–100 cm (Fig. 1A and 1B).

Over 10-year of historical cropping sequences, the significant responses of SOC stock and its distribution varied depending on the tillage and cropping systems (Table 3). SOC stock under NT-Mz crop rotation systems increased by 50%, 26%, and 15%, equivalent to a gain of 4.6, 2.6, and 2.2 Mg C ha^{-1} at 0–5, 5–10, and 10–20 cm depth, respectively. Unlike SOC concentration, NT1-Mz only significantly increased SOC stock in the top soils by 68% and 26%, equivalent to 6.1 and 2.2 Mg C ha^{-1} in the 0–5 and 5–10 cm, respectively. Despite CT-Mz significantly accumulated SOC stock in the tilled layers (5–10 and 10–20 cm), significant reductions were found in the 60–80 and 80–100 cm depths (Table 3).

At the first sampling in 2011, SOC stock in 0–20 cm depth under RV was significantly ($P < 0.05$) higher at 17%, 21%, and 22% than under CT-Mz, NT1-Mz, and NT-Mz crop rotation systems, respectively. In 2021, SOC stock under CT-Mz and all the NT-Mz did not differ ($P < 0.05$) from RV. SOC stock was fully recovered under NT-Mz crop rotation systems and even surpassed the RV's stock by +0.64 Mg C ha^{-1} under NT1-Mz, while the difference with RV decreased from 17 to 8% under CT-Mz (Fig. 3A).

Considering a 100 cm as a single stratum, all the NT-Mz cropping systems significantly increased SOC stock, with accumulation rates ranging from 0.86 to 1.47 Mg C ha^{-1} yr^{-1}, while it was not the case in CT-Mz (Table 3).

Over 10 years of cultivation, all the NT-Mz systems significantly increased N concentration in the top 0–5 cm depth (32% in NT-Mz crop rotation systems and 44% in NT1-Mz), but soil N significantly decreased by -24% in NT1-Mz at 40–60 cm and at least from -18 to -21% in NT-Mz crop rotation systems from 40 to 100 cm. In CT-Mz, soil N concentration remained neutral from 0 to 40 cm, then significantly decreased in the 40–60 and 80–100 cm depths (Fig. 2A and 2B).

N stock in the soils under all the NT-Mz systems significantly increased in the uppermost soil surface (0–5 cm), remained neutral in the 5–40 cm, then significantly decreased below 40 cm (Table 4). Averaged N stock in NT-Mz crop rotation systems increased by 32%, equivalent to 0.3 Mg N ha^{-1} in the 0–5 cm, whereas there was significant reduction of -16% and -23% from 40 to 100 cm. In NT1-Mz, N stock significantly increased in the 0–5 cm layer by 43%, equivalent to 0.4 Mg N ha^{-1}, then significantly reduced by -23% in the 40–60 cm. In the case of CT-Mz, N stock remained constant from 0–40 cm, then significantly declined from -14 to -24% from 40 to 100 cm (Table 4).

When RV was used as a reference (0-20 cm depth), N stock in 2011 was significantly lower by 25%, 22%, and 20% under CT-Mz, NT1-Mz, and NT-Mz crop rotation systems, respectively. In 2021, the difference in N stock decreased to 15% and 7.5% under the NT1-Mz and NT-Mz crop rotation systems, respectively, when compared with RV. In contrast to NT-Mz, N stock did not change under CT-Mz in 2021 (Fig. 3D).

Although not significant, after nearly a decade of rice-based and the recent shift to maize-based systems, soil N stock in the whole profile (0–100 cm) showed a decreasing trend by -11%, -6%, and -5%, equivalent to annual loss rates of -0.11, -0.06, and -0.06 Mg N ha^{-1} yr^{-1} under CT-Mz, NT1-Mz, and NT-Mz biannual rotation systems, respectively (Table 4).

3.1.2 Soybean-based experiment

After 10 years of implementation, significant increase ($p < 0.05$) in SOC concentration was only observed under all the NT-Sb cropping systems (NT1-Sb, NT2-Sb, and NT3-Sb) in the top 0–5 cm with a similar increase amount of ~7.5 g C kg^{-1} soil. SOC concentration in CT-Cs remained stable across the whole profile (Fig. 1C and 1D).

SOC stock significantly increased in the surface layers (0–10 cm) under all NT-Sb systems (Table 3). SOC stock significantly increased by 3.6 Mg C ha-1 in the 0–5 cm in NT1-Sb. NT-Sb crop rotation systems (average of NT2-Sb and NT3-Sb) significantly accumulated SOC stock by 3.55 and 1.75 Mg C ha^{-1} in 0–5 and 5–10 cm, respectively, along with a positive trend from 10 to 60 cm depth. Unlike all the NT-Sb systems, the SOC stock in the CT-Sb soil remained neutral across the whole profile (Table 3).

In 2011, RV as a reference (0-20 cm) significantly stored 13%, 14%, and 16% higher SOC stock than CT-Sb, NT1-Sb, and NT-Sb bi-annual rotation, respectively. In 2021, SOC stock increased and a 100% recovery was observed under NT-Sb biannual rotation systems, while the differences with RV dropped to 9% and 5% under CT-Sb and NT1-Sb, respectively, although not significant (Fig. 3B).

Considering the whole profile (0–100 cm), after 10 years of cultivation, the study showed that all the NT-Sb systems increased SOC stock with annual accumulation rates ranging from 0.65 to 1.00 Mg C ha^{-1} yr^{-1} although non-significant difference was detected (Table 3).

From 2011 to 2021, all the NT-Sb systems significantly increased soil N concentration in the top 0–5 cm with a similar increase of ~0.46 g N kg^{-1} soil (Fig. 2C and 2D). Noticeably, significant decreases in N concentration were observed in the 40–60, 60–80, and 80–100 cm under NT-Sb bi-annual rotation systems and in the 60–80 and 80–100 cm depths under NT1-Sb (Fig. 2C and 2D). In contrast to NT-Sb systems, N concentration remained constant across the whole profile in soybean monocropping under conventional plough-based tillage (CT-Sb) (Fig 2D).

Over 10 years of cultivation, the N stock significantly increased in a similar amount of 0.2 Mg N ha^{-1} under all the NT-Sb systems in the top 0–5 cm. The N stock under all the NT-Sb systems remained stable from 5 to 60 cm, then significantly decreased in the 60–80 and 80–100 cm depths (Table 4).

In 2011, RV's soil (0-20 cm) significantly stored 23%, 20%, and 18% higher N stock than CT-Sb, NT1-Sb, and NT-Sb crop rotation systems, respectively. In 2021, N stock increased and the differences with RV reduced to 16%, 18%, and 11% under CT-Sb, NT1-Sb, and NT-Sb crop rotation systems, respectively, although not significant (Fig. 3E).

Measured in the whole profile (0–100 cm), soybean monocropping under NT systems (NT1-Sb) significantly decreased N stock with an annual loss rate of -0.1 Mg N ha^{-1} yr^{-1}, while NT-Sb bi-annual rotation systems exhibited a decrease trend of N stock with an annual loss rate of -0.06 Mg N ha^{-1} yr^{-1} (Table 4). Despite the significant increase in the 10–20 and decrease in the 60–80 cm, N stock in CT-Sb remained stable when considering the whole profile 0–100 cm (Table 4).

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

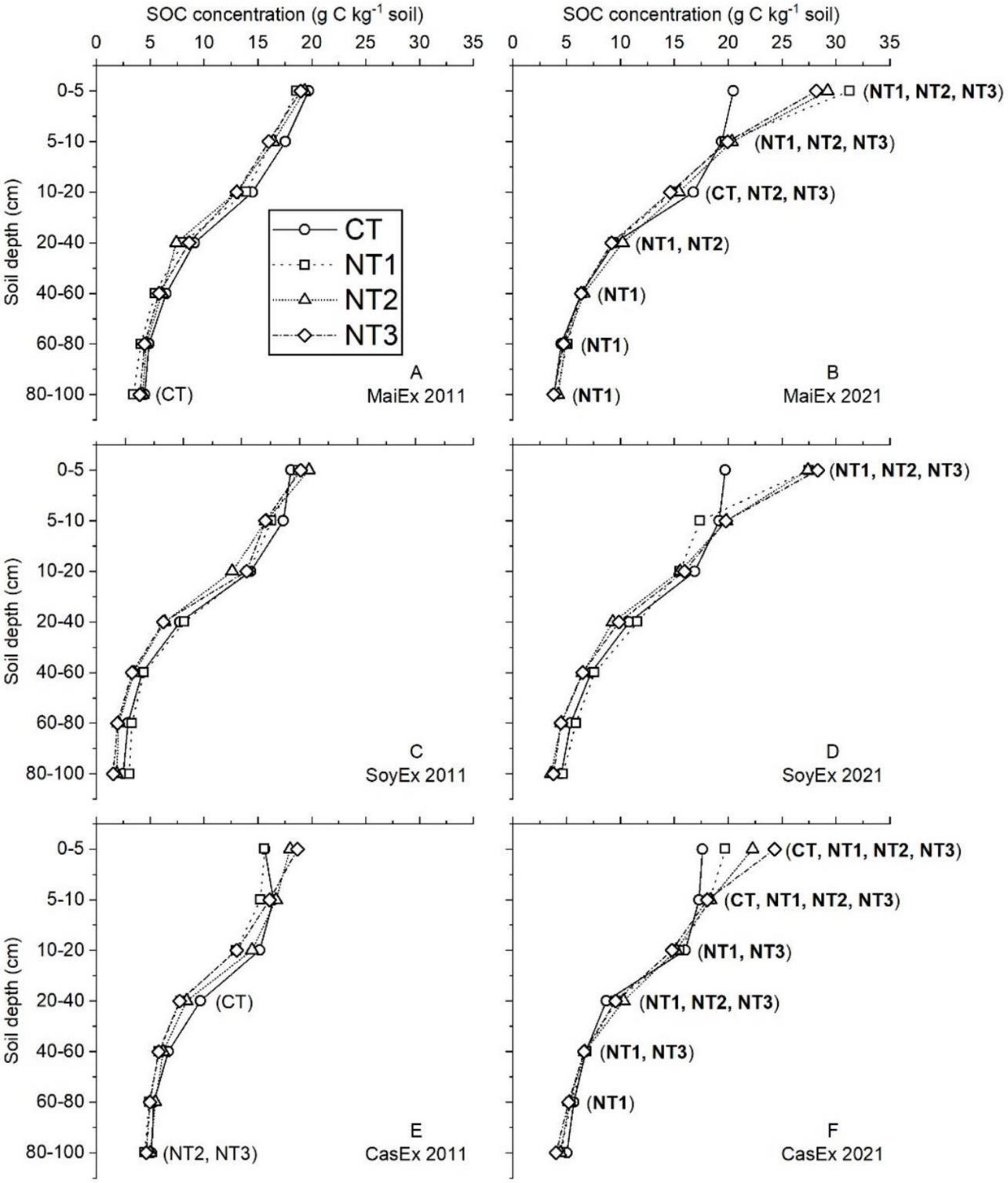

Figure 1. SOC concentration (0-100 cm) in 2011 and 2021 under different cropping systems. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with different cropping systems as described in Table 1. Treatment(s) in bold within the brackets indicate the gain and significant difference in concentrations between 2011 and 2021 in the same treatment at the same soil depth.

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Preprint. Discussion started: 7 March 2024

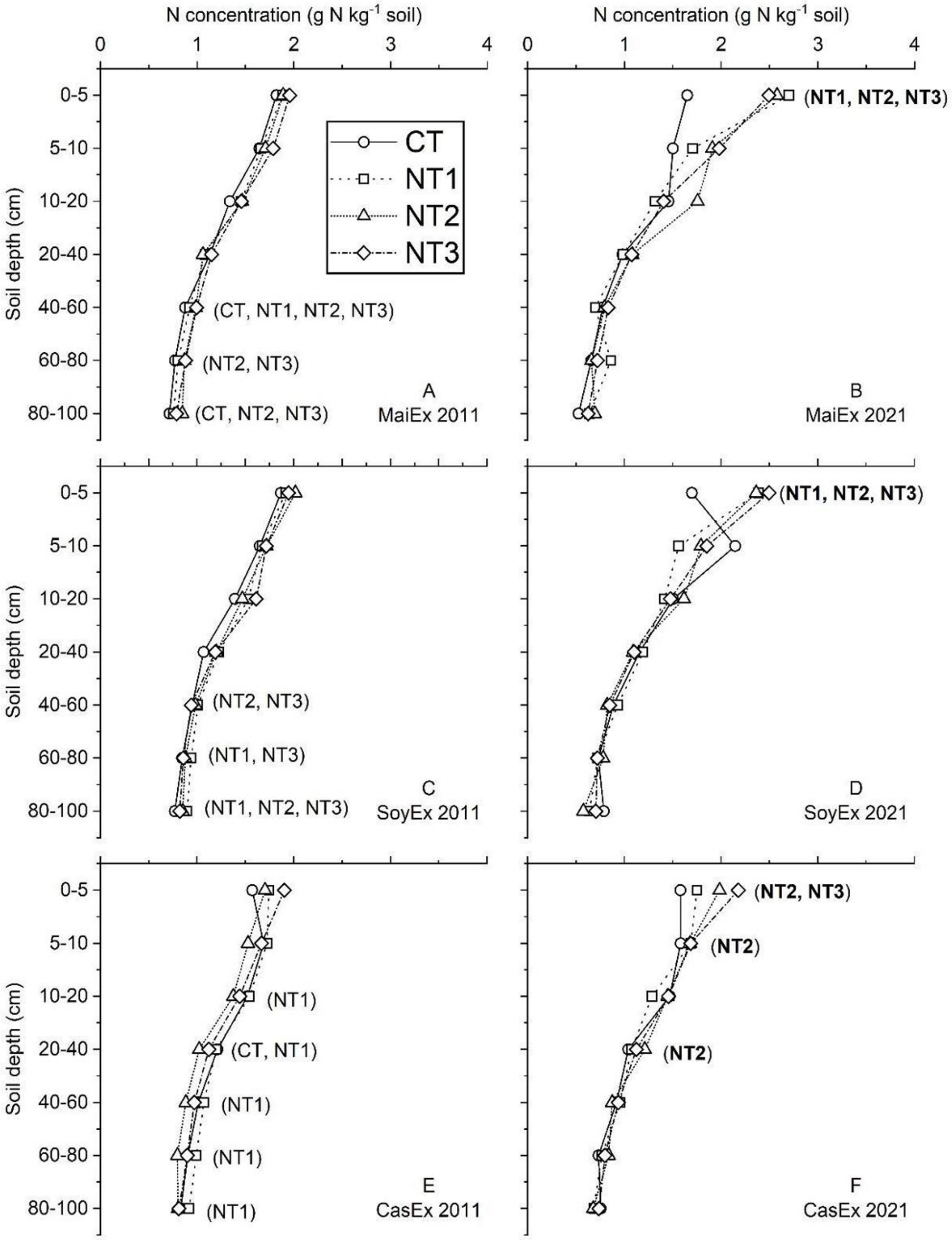

Figure 2. Total N concentration (0-100 cm) in 2011 and 2021 under different cropping systems. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with different cropping systems as described in Table 1. Treatment(s) in bold within the brackets indicate the gain and significant difference in concentrations between 2011 and 2021 in the same treatment at the same soil depth.

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

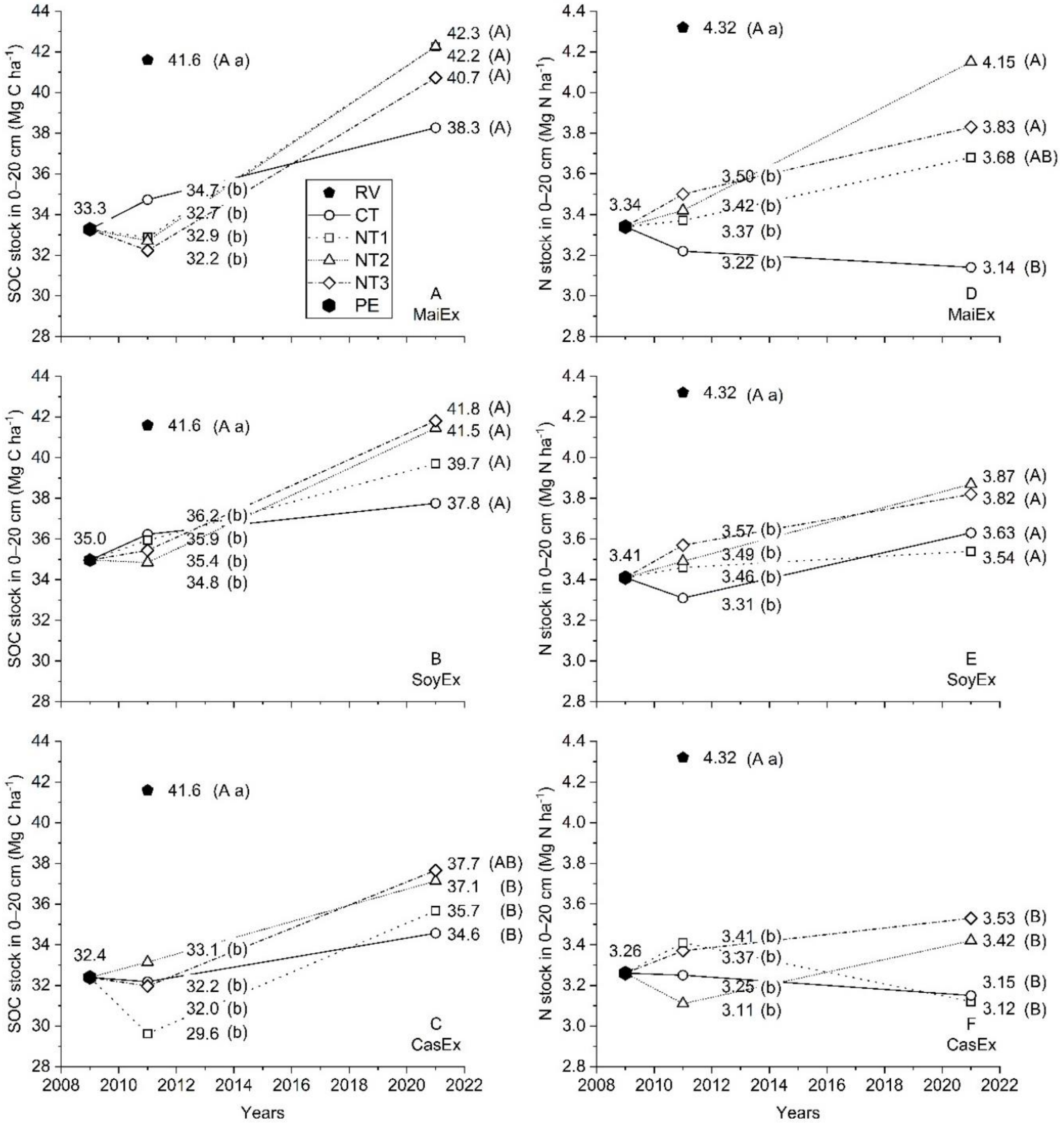

Figure 3. Changes in SOC and N stocks (Mg ha-1) at 0−20 cm depth from pre-experiment (PE) in 2009, 2011, and 2021 under different cropping systems with reference vegetation (RV) for comparison. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with different cropping systems as described in Table 1. Lowercase letters inside the brackets indicate significant difference between RV and the treatment(s) in 2011 and Uppercase letters inside the brackets indicate significant difference between RV and the treatment(s) in 2021 (Tukey's test; $P < 0.05$).

3.1.3 Cassava-based experiment

Over a decade, all the treatments significantly ($P < 0.05$) increased SOC concentration in the upper layers (0–10 cm), while significant increases below 10 cm were only observed under NT-Cs systems (Fig. 1F). Among the two treatments of NT-Cs crop rotation systems (NT2-Cs and NT3-Cs), NT3-Cs significantly increased SOC concentration from the top 0 to 60 cm by 30%, 12%, 13%, 23%, and 15% in the 0–5, 5–10, 10–20, 20–40, and 40–60 cm, respectively, while a significant decrease of -13% was recorded in the 80–100 cm depth. Under NT2-Cs, significant increases in SOC concentration were observed up to 40 cm depth with a gain of 24%, 10%, and 23% in 0–5, 5–10, and 20–40 cm, respectively, with a significant decrease by -12% in 80–100 cm depth (Fig. 1E and 1F). In the case of cassava monocropping under NT systems (NT1-Cs), significant increases in SOC concentration were detected from 0 to 80 cm with a gain of 26%, 20%, 19%, 22%, 18%, and 10% in the 0–5, 5–10, 10–20, 20–40, 40–60, and 60–80 cm, respectively (Fig. 1F). For CT-Cs, SOC concentration significantly increased, but at 2 times lower than that of those NT-Cs systems, by 12% and 5% in the top 0–5 and 5–10 cm, respectively, with a significant decrease by -10% detected in 20–40 cm depth (Fig. 1E and 1F).

From 2011 to 2021, SOC stocks under the NT-Cs crop rotation systems (NT2-Cs and NT3-Cs) increased significantly ($P < 0.05$) by 2.4, 1.1, 1.4, and 2.9 Mg C ha^{-1} in 0–5, 5–10, 10–20, and 20–40 cm, respectively, with still a positive trend in 40-60 cm and then a significant decrease by -0.9 Mg C ha^{-1} in 80–100 cm (Table 3). Similarly, NT1-Cs significantly increased SOC stock by 2.0, 1.6, 2.5, and 2.2 Mg C ha^{-1} in 0–5, 5–10, 10–20, and 20–40 cm, respectively (Table 3). For cassava monocropping under conventional tillage (CT-Cs), SOC stock significantly increased, but at 2 times lower than all the NT-Cs systems, by 0.9, 0.5, and 0.9 Mg C ha^{-1} in the 0–5, 5–10, and 10–20 cm, respectively, while no significant changes were recorded below 40 cm (Table 3).

In 2011, with RV used as a reference (0–20 cm), SOC stock was significantly higher in RV by 23%, 29%, and 22% in CT-Cs, NT1-Cs, and NT-Cs crop rotation systems (average of NT2-Cs and NT3-Cs), respectively. In 2021, the difference in SOC stock with RV declined to 17%, 14%, and 10% in CT-Cs, NT1-Cs, and NT-Cs crop rotation systems, respectively (Fig. 3C).

Over a 10-year period, from 0 to 100 cm depth, SOC stock significantly increased under all the NT-Cs systems, with an accumulation rate ranging from 0.70 to 1.07 Mg C ha^{-1} yr^{-1}, while the SOC stock change in CT-Cs was not significant and remain stable (Table 3).

Surprisingly, the response of soil N concentration to tillage systems differed from SOC. NT-Cs crop rotation systems (NT2-Cs and NT3-Cs) significantly increase soil N in the uppermost layer (0–5 cm) with an average increase of 16% (0.28 g N kg^{-1} soil), whereas significant increases were observed by 10% in 5–10 cm and by 19% in 20–40 cm under NT2-Cs (Fig. 2F). Over a 10-year period, cassava monocropping under NT systems (NT1-Cs) resulted in stable N concentration in the top 0–10 cm, but the concentration significantly decreased below 10 cm by -10 to -25% from 10 to 100 cm depth (Fig. 2E). For CT-Cs, N concentration remained stable throughout the soil profile, except a significant loss of -14% detected in the 20–40 cm layer (Fig. 2E).

In the case of N stock, among the two NT-Cs crop rotation systems (NT2-Cs and NT3-Cs), NT3-Cs significantly increased N stock by 15% (0.1 Mg N ha^{-1}) in the surface 0–5 cm, while significant increases were found by 0.1, 0.1 and 0.3 Mg N ha^{-1} at 0–5, 5–10, and 20–40 cm, respectively, in the NT2-Cs (Table 4). In the case of cassava monocropping, N stock in the NT1-Cs soil remained constant in the top 0–10 cm, then significantly decreased from 10 to 100 cm from -0.3 to -0.5 Mg N ha^{-1}. In a similar trend to NT1-Cs, N stock in CT-Cs soil did not change at the top 0–20 cm, but significantly decreased from -0.2 to -0.3 Mg N ha^{-1} from 20 to 100 cm (Table 4).

From 2011 to 2021, considering 0–20 cm depth, all tillage and cropping systems did not alter N stocks when compared to RV (Fig. 3F).

When considering the whole profile (0–100 cm), N stock in the NT-Cs bi-annual rotation systems did not change with time. Regardless of tillage systems, long-term cassava monocropping resulted in significant decreases of N with an annual depletion rate of -0.11 and -0.17 Mg N ha^{-1} yr^{-1} under CT-Cs and NT1-Cs, respectively (Table 4).

Table 3. Mean SOC stocks in 2021 and SOC stock change rate between 2021 and 2011.

Experiments[a]	**Approximate soil depth (cm)**	**Cropping systems**[b]							
		CT	**NT1**	**NT2**	**NT3**	**CT**	**NT1**	**NT2**	**NT3**
		SOC stock in 2021 (Mg C ha^{-1})				**SOC stock change rate 2021-11 (Mg C ha^{-1} yr^{-1})**			
MaiEx	0-5	9.83 c	15.01 Aa	14.03 Aab	13.52 Ab	0.04	**0.61**	**0.48**	**0.44**
	5-10	10.16 Ab	10.91 Aab	11.16 Aa	10.89 Aab	**0.09**	**0.22**	**0.23**	**0.22**
	10-20	18.28 A	16.31	17.08 A	16.32 A	**0.23**	0.11	**0.25**	**0.19**
	20-40	19.90	18.61	20.28 A	18.64	0.11	0.16	**0.45**	0.07
	40-60	12.58	12.18	12.75	12.31	-0.04	0.08	0.10	0.02
	60-80	9.01 B	9.58	9.64	9.33	**-0.12**	0.07	-0.02	-0.02
	80-100	7.17 B	7.51	7.97	7.26	**-0.13**	0.05	-0.02	-0.06
	0-100	86.92	90.13 A	92.90 A	88.27 A	0.17	**1.30**	**1.47**	**0.86**
SoyEx	0-5	9.45 b	13.17 Aa	13.14 Aa	13.58 Aa	0.02	**0.36**	**0.32**	**0.39**
	5-10	9.95	9.74	11.05 A	10.80 A	0.03	0.04	**0.18**	**0.17**
	10-20	18.35	16.79	17.27	17.43	0.11	-0.02	0.17	0.08
	20-40	22.34	22.66	19.26	19.69	0.19	0.17	0.19	0.18
	40-60	15.20	15.86	13.00	13.08	0.14	0.10	0.13	0.17
	60-80	11.16	11.95	9.48	9.32	0.04	0.06	0.01	0.04
	80-100	8.76	9.37	7.03	7.25	-0.04	-0.05	-0.08	-0.02
	0-100	95.22	99.53	90.23	91.15	0.48	0.65	0.92	1.00
CasEx	0-5	8.44 Ac	9.48 Ab	10.68 Aa	11.67 Aa	**0.09**	**0.20**	**0.21**	**0.27**
	5-10	8.97 Ab	9.52 Aab	9.82 Aa	9.76 Aa	**0.05**	**0.16**	**0.10**	**0.12**
	10-20	17.15 A	16.66 A	16.63 A	16.23 A	**0.09**	**0.25**	**0.09**	**0.18**
	20-40	18.18	18.84 A	20.77 A	19.27 A	-0.09	**0.22**	**0.29**	**0.29**
	40-60	13.12	13.42	13.95	12.99	-0.05	0.14	0.12	0.13
	60-80	11.11	10.78	10.61	10.18	0.02	0.09	-0.01	0.04
	80-100	9.47	8.71	8.62 B	7.91 B	0.02	0.01	**-0.10**	**-0.08**
	0-100	86.44	87.42 A	91.07 A	88.01 A	0.14	**1.07**	**0.70**	**0.95**

[a] MaiEx: Maize-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment.

[b] CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with cropping systems as described in Table 1. Different uppercase letters accompanying the values indicate significant difference within the same treatment between 2011 and 2021 (diachronic), and different lowercase letters indicate significant difference between the treatments within the same sampling date (synchronic) at the same soil depth at $P \leq 0.05$ (Tukey's test). Values of SOC stock change rates in bold indicate changes for a given treatment and depth between 2021 and 2011 are significantly different from 0 at $P \leq 0.05$ (Tukey's test). Positive values of SOC stock change rate indicate a SOC accumulation; negative values indicate a SOC loss.

Table 4. Mean total N stock in 2021 and N stock change rate between 2021 and 2011.

Experiments[a]	Approximate soil depth (cm)	Cropping systems[b]							
		CT	NT1	NT2	NT3	CT	NT1	NT2	NT3
		N stock in 2021 (Mg N ha^{-1})				N stock change rate 2021-11 (Mg N ha^{-1} yr^{-1})			
MaiEx	0-5	0.79 b	1.30 Aa	1.24 Aa	1.20 Aa	-0.01	**0.04**	**0.03**	**0.03**
	5-10	0.79 b	0.95 ab	1.04 a	1.06 a	-0.01	0.01	0.01	0.01
	10-20	1.55 ab	1.44 b	1.87 a	1.58 ab	0.01	-0.01	0.03	0.00
	20-40	1.98	1.91	2.18	2.11	-0.02	-0.02	0.01	-0.02
	40-60	1.46 B	1.33 B	1.48 B	1.56 B	**-0.02**	**-0.04**	**-0.03**	**-0.03**
	60-80	1.24 B	1.49	1.25 B	1.36 B	**-0.02**	-0.01	**-0.04**	**-0.04**
	80-100	1.00 B	1.22	1.21 B	1.17 B	**-0.03**	-0.02	**-0.03**	**-0.04**
	0-100	8.82	9.63	10.27	10.03	-0.11	-0.06	-0.03	-0.09
SoyEx	0-5	0.82 b	1.15 Aa	1.13 Aa	1.20 Aa	-0.01	**0.02**	**0.02**	**0.03**
	5-10	1.07	0.87	0.99	1.00	0.02	0.00	0.01	0.01
	10-20	1.75 A	1.53	1.74	1.62	**0.02**	-0.01	0.01	-0.01
	20-40	2.28	2.29	2.20	2.14	0.02	-0.01	-0.01	-0.02
	40-60	1.74	1.80 A	1.60	1.62	0.00	-0.02	-0.03	-0.02
	60-80	1.44 B	1.47 B	1.44 B	1.39 B	**-0.02**	**-0.03**	**-0.02**	**-0.02**
	80-100	1.36	1.21 B	1.18 B	1.26 B	-0.01	**-0.04**	**-0.04**	**-0.02**
	0-100	10.45	10.31 B	10.29	10.23	0.02	**-0.10**	-0.06	-0.06
CasEx	0-5	0.76 c	0.84 bc	0.95 Aab	1.05 Aa	0.00	0.00	**0.01**	**0.01**
	5-10	0.82	0.88	0.89 A	0.91	0.00	0.00	**0.01**	0.00
	10-20	1.57 ab	1.40 Bb	1.57 ab	1.58 a	-0.01	**-0.03**	0.01	0.00
	20-40	2.06 B	2.04 B	2.34 A	2.19	**-0.03**	**-0.03**	**0.03**	0.00
	40-60	1.69 B	1.74 B	1.75	1.74	**-0.02**	**-0.03**	0.01	-0.01
	60-80	1.48 B	1.51 B	1.54	1.52	**-0.02**	**-0.04**	0.00	-0.02
	80-100	1.32	1.29 B	1.34	1.35	**-0.02**	**-0.05**	-0.01	-0.02
	0-100	9.70 B	9.70 B	10.40	10.34	**-0.11**	**-0.17**	0.06	-0.03

[a] MaiEx: Maize-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment.

[b] CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with cropping systems as described in Table 1. Different uppercase letters accompanying the values indicate significant difference within the same treatment between 2011 and 2021 (diachronic), and different lowercase letters indicate significant difference between the treatments within the same sampling date (synchronic) at the same soil depth at $P \leq 0.05$ (Tukey's test). Values of N stock change rates in bold indicate changes for a given treatment and depth between 2021 and 2011 are significantly different from 0 at $P \leq 0.05$ (Tukey's test). Positive values of N stock change rate indicate a N accumulation; negative values indicate a N loss.

https://doi.org/10.5194/egusphere-2024-541
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3.2 The impact of cropping systems on organic C and N concentrations in size fractions

Fig. 4 (with duplicates of Table S5 and S6 in supplementary materials) shows the depth distribution and the proportion of C in POM (C-POM) and MAOM (C-MAOM) concentrations in 2011 and 2021, while the depth distribution and the proportion of N concentrations in the POM (N-POM) and MAOM (N-MAOM) fractions in 2011 and 2021 are presented in Fig. 5 (with the duplicates in Table S7 and S8 in the supplementary materials).

3.2.1 Maize-based experiment

Over the 10-year period, the concentrations of C-POM and C-MAOM were significantly ($P < 0.05$) influenced by all the treatments; however, the effects varied across cropping systems (Fig. 4A and 4B). Among the two treatments of NT-Mz crop rotation systems (NT2-Mz and NT3-Mz), NT3-Mz significantly increased C-POM by 126% in 0–5 cm and C-MAOM by 45% and 19% in 0–5, and 5–10 cm, respectively. NT2-Mz significantly increased C-POM by 117%, 48%, and 68% and C-MAOM by 43%, 21%, and 37% in the 0–5, 5–10, and 20–40 cm, respectively (Fig. 4A and 4B). For NT1-Mz, significant increases were found in C-POM by 226% and 59% in 0–5 and 5–10 cm depths, respectively. C-MAOM concentration was also significantly increased under NT1-Mz by 50%, 17%, 14%, and 12% in 0–5, 5–10, 20–40, and 40–60 cm, respectively (Fig. 4A and 4B). Significant increases in C-POM by 44% and 52% and C-MAOM by 14% and 19% in the 5–10 and 10–20 cm, respectively, were observed in CT-Mz soil (Fig. 4A and 4B). At 80–100 cm depth, a significant decrease in C-MAOM by -11%, was observed under CT-Mz, while this was not the case for all the NT-Mz systems (Fig. 4A and 4B).

Adoption of all the NT-Mz systems significantly ($P < 0.05$) increased N-POM concentration in the surface layers (0–10 cm), while significant decreases were observed below 40 cm (Fig. 5A and 5B). The concentration of N-POM under NT3-Mz significantly increased by 151% in 0–5 cm, but significantly decreased by -41% in 40–60 cm layer. NT2-Mz significantly increased N-POM by 142% and 77% in 0–5 and 5–10 cm, respectively, whereas the concentration was significantly reduced by -42% to -76% from 40 to 100 cm depths (Fig. 5A and 5B). The N-POM concentration was significantly increased in NT1-Mz by 258% and 61% in the top 0–5 and 5–10 cm, respectively, but was significantly reduced by -26% and -71% in 40–60 and 80–100 cm depth, respectively. N-POM under CT-Mz did not change in the surface soils but significantly decreased by -59% to -77% from 40 to 100 cm (Fig. 5A and 5B). Our diachronic study showed that adopting crop rotations under NT-Mz systems did not enhance N-MOAM at any soil depth but resulted in significant ($P < 0.05$) N loss in MAOM below 5 cm in NT2-Mz and below 20 cm in NT3-Mz (Fig. 5A and 5B). For rice monocropping for nearly a decade and the shift to recent maize monocropping under both conventional tillage and NT systems, the concentration of N-MAOM remained stable throughout the whole profile, with the exception of significant ($P < 0.05$) decreases found in 40–60 and 80–100 cm depths under CT-Mz and NT1-Mz, respectively (Fig. 5A and 5B).

3.2.2 Soybean-based experiment

Under this diachronic analysis, the concentration of C and N in the POM fraction showed similar patterns and magnitudes as in MaiEx (Fig. 4 and 5). C-POM in the top 0–20 cm was influenced by tillage and cropping systems. Adoption of NT-Sb crop rotation systems (NT2-Sb and NT3-Sb) significantly ($P < 0.05$) increased C-POM by 115% and 47% in 0–5 and 5–10 cm, respectively (Fig. 4D). Under NT1-Sb, C-POM significantly increased by 215%, 101% and 72% in 0–5, 5–10, and 10–20 cm, respectively. The amount of C-POM in CT-Sb soil significantly increased in the ploughed layers (5–10 and 10–20 cm) but at approximately 2 times lower than those NT-Sb systems (Fig. 4D). The C-POM concentration did not change below 20 cm in all the treatments, with the exception of a significant decrease under NT3-Sb in 80–100 cm (Fig. 4C and 4D). The effects of cropping systems on C-MAOM concentration varied across the four treatments and soil depths (Fig. 4C and 4D). Compared between the two treatments in NT-Sb crop rotation systems, NT3-Sb significantly ($P < 0.05$) enhanced C-MAOM by 25%, 29%, 7%, and 13% in 0–5, 5–10, 10–20, 40–60 cm depth, respectively. In the case of NT2-Sb, C-MAOM significantly increased by 22% and 8% in 0–5, and 10–20 cm, respectively, with a significant reduction by -19% in the lowest profile (80–100 cm). Similar to NT2-Sb, NT1-Sb significantly increased C-MAOM by 28% and 5% in 0–5 and 10–20 cm, respectively, with a significant decline by -16% detected in the lowest profile (80–100 cm). For CT-Sb, significant increases in C-MAOM were observed by 7% and 12% in 10–20 and 40–60 cm depth, respectively, with a significant decrease by -13% spotted in 80–100 cm depth (Fig. 4C and 4D).

From 2011 to 2021, the amount of N-POM fraction under NT-Sb crop rotation systems significantly ($P < 0.05$) increased by an average of 0.13 g N kg^{-1} soil at 0–5 cm and 0.02 g N kg^{-1} soil at 5–10 cm. Significant decrease of N-POM below 60 cm was found in NT3-Sb, but it was not the case in NT2-Sb (Fig. 5D). NT1-Sb significantly increased N-POM by 0.21, 0.05, and 0.02 g N kg^{-1} soil in 0–5, 5–10, and 10–20 cm, respectively, while the concentration remained constant below 20 cm. Under CT-Sb, N-POM significantly increased at the same amount of 0.02 g N kg^{-1} soil in the plough layers (5–10 and 10–20 cm), with a significant decrease found in 80–100 cm (Fig. 5C and 5D).

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

In contrast to C-POM, the amount of N- MAOM in all the NT-Sb systems did not change from 0 to 40 cm, but significant reductions were observed below 40 cm. NT2-Sb significant loss N- MAOM by -43% to -61% from 60 to 100 cm, whereas a significant loss of 40% at 80–100 cm was observed in NT3-Sb (Fig. 5C and 5D). Under long-term soybean monocropping, the concentration of N-MAOM significantly decreased by -25% to -49% and -33% to -58% from 60–100 cm under NT1-Sb and CT-Sb, respectively (Fig. 5C and 5D).

3.2.3 Cassava-based experiment

After 10 years of experimentation, all the treatments in CasEx had no significant effect on the C-POM concentration in the topsoil (0–20 cm) as observed in MaiEx and SoyEx (Fig. 4E and 4F). Except for a few significant increases in C-POM detected under NT1-Cs and NT3-Sb in 20–40 cm and significant decreases in C-POM under CT-Cs and NT2-Cs in 20–40 and 60–80 cm, respectively, the C-POM concentration in all treatments remained constant below 20 cm (Fig. 4E and 4F). Surprisingly, all the treatments had a significant positive impact on C-MAOM in the upper 0–20 cm, and the effects even extended below 20 cm under all the NT-Cs systems (Fig. 4E and 4F). The concentration of C-MAOM significantly increased under NT-Cs crop rotation systems (average of NT2-Cs and NT3-Cs) by 26%, 11%, 9%, 24%, and 13% in 0–5, 5–10, 10–20, 20–40, and 40–60 cm, respectively. NT1-Cs significantly enhanced C-MAOM by at least 11% to 23% from 0 to 80 cm (Fig. 4E). The concentration of C-MAOM was significantly increased under CT-Cs at roughly 6% in the top 0–20 cm, and the concentration did not change below 20 cm (Fig. 4E and 4F).

https://doi.org/10.5194/egusphere-2024-541
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Over a 10-year period, the concentration of N-POM was not influenced by any treatments, while all the treatments significantly reduced the N-MAOM concentration across the soil profile (Fig. 5E and 5F). Despite the fact that the N-MAOM did not change in the top 0–5 cm, the concentration significantly decreased by -14% to -69% from 5 to 100 cm across all three NT-Cs systems. For CT-Cs, N-MAOM concentration significantly decreased in the whole profile (0–100 cm) with a depletion of -20% to -52% (Fig. 5E and 5F).

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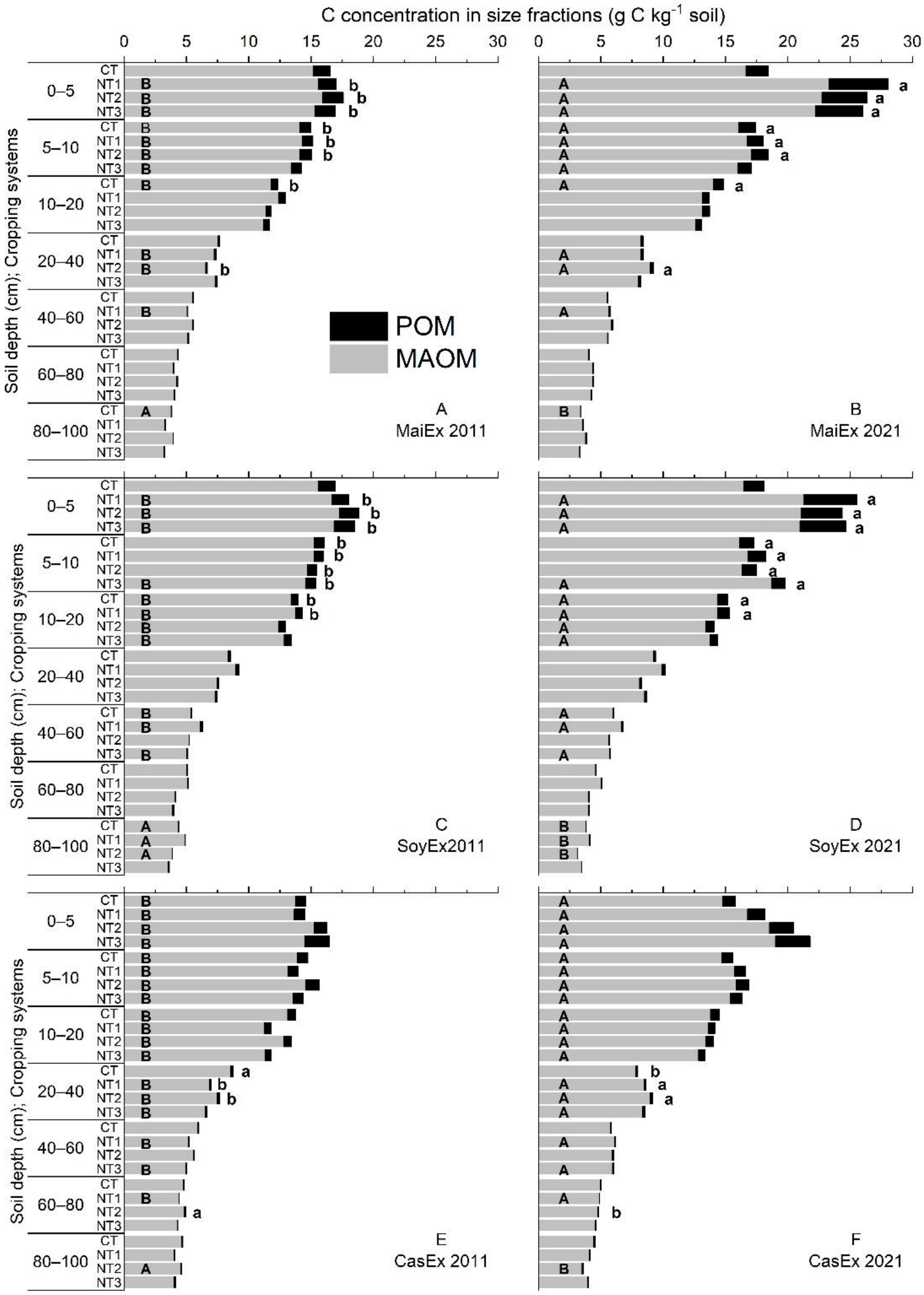

Figure 4. Amount of C in mineral-associated organic matter (MAOM) and particulate organic matter (POM) fractions 2011 and 2021 under different cropping systems. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till mulch-based cropping systems associated with different cropping systems as described in Table 1. Uppercase letters on the bars and lowercase letters in front of the bars indicate a significant difference (Tukey's test; P < 0.05) in C concentration in MAOM and POM, respectively, between 2011 and 2021 for the same treatment and soil depth.

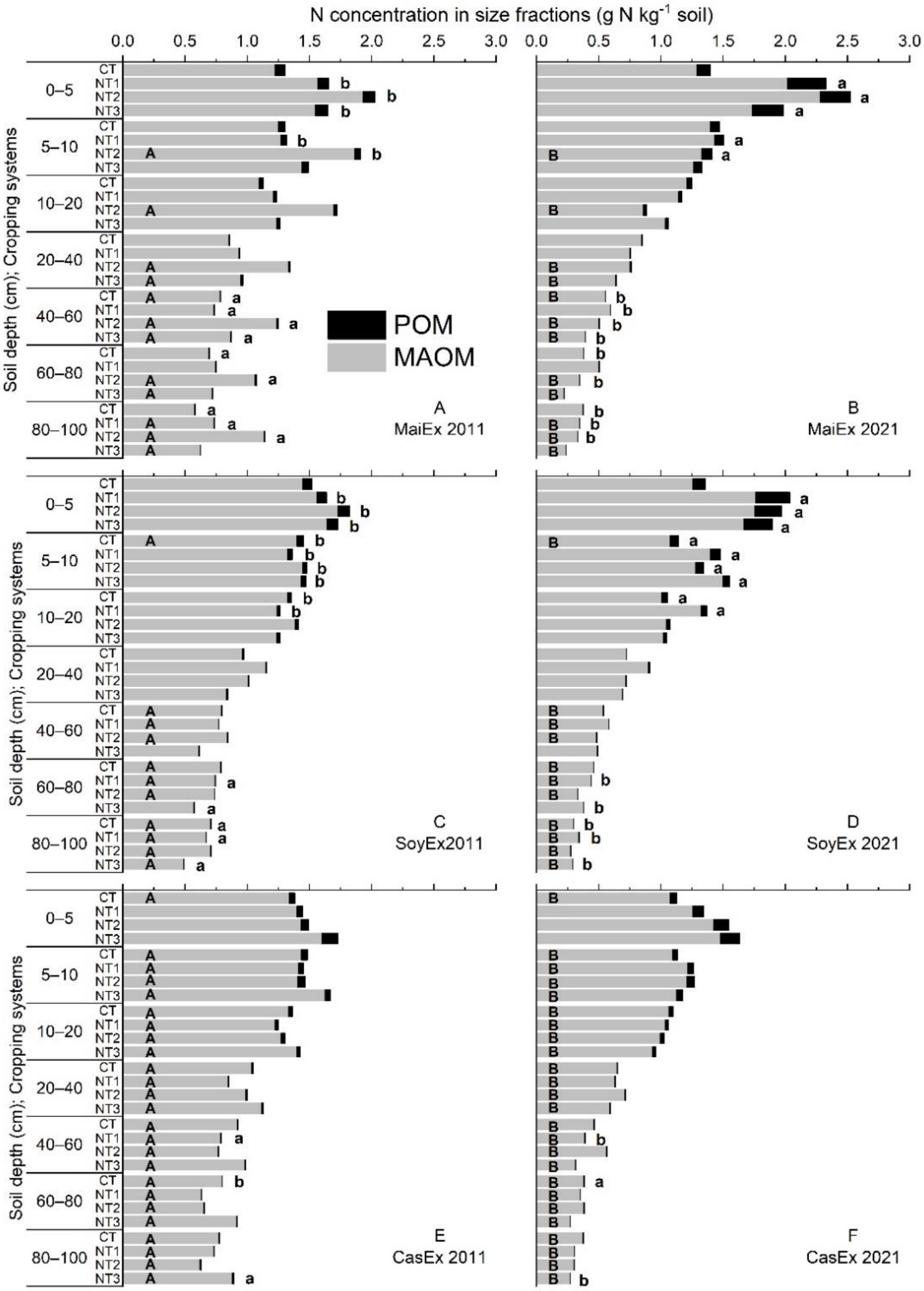

Figure 5. Amount of N in mineral-associated organic matter (MAOM) and particulate organic matter (POM) fractions in 2011 and 2021 under different cropping systems. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till mulch-based cropping systems associated with different cropping systems described in Table 1. Uppercase letters on the bars and lowercase letters in front of the bars indicate a significant difference (Tukey's test; $P < 0.05$) in N concentration in MAOM and POM, respectively, between 2011 and 2021 for the same treatment and soil depth.

4 Discussion

4.1 Change in SOC stock

This study quantified the impacts of cropping systems on changes in SOC and N stocks and their fractions down to 100 cm depth in three long-term annual crop production experiments. Over 10 years, NT systems modified the SOC stock and its vertical distribution (Table 3).

Several studies reported that long-term NT adoption accumulated SOC stock only on the surface soils, but the stock did not differ from CT when considering the whole soil profile (Blanco-Canqui and Lal, 2008; Luo et al., 2010; Blanco-Canqui et al., 2011; Du et al., 2017; Xiao et al., 2020). For example, a recent meta-analysis from 86 studies covering a range of crop productions across the world, (Xiao et al., 2020) found that NT systems significantly accumulated the SOC stock only in the top 0–5 cm, and no significant change was found below 5 cm. Across climatic conditions, soil types, and various cropping systems in China, based on 95 comparisons between NT and CT, adopting NT led to increase in SOC stock by 3.8% in the upper 20 cm layer but to a decrease in SOC in the 30–40 cm layer (Du et al., 2017). Similarly, from a systematic review of global data of 69 paired-experiments, (Luo et al., 2010) reported that long-term NT adoption only significantly affected SOC stock in the top 0–10 cm but not down to 40 cm depth. The authors also reported that increasing crop species diversity resulted in a lower SOC accumulation in the surface and a greater SOC loss in deeper layers.

SOC stock changes reported under NT systems may differ according to climate, soil type and cropping systems (Paustian et al., 1997; Six et al., 2002; Bayer et al., 2006; Ogle et al., 2012; Virto et al., 2012).

Soils in the tropical climate require diversified and large amounts of C inputs for NT viability due to fast residue decomposition and difficulty in maintaining soil cover (Séguy et al., 2006; Castro et al., 2015). In the same experiments as in our study, Hok et al. (2015) reported that NT systems with diverse crop species significantly accumulated SOC at the surface 0–5 cm after 4 years of NT adoption. Although there is variability in SOC stock accumulation and its vertical distribution among the three NT systems, our results revealed that NT systems significantly increased SOC stock with accumulation rate ranging from 0.38 to 0.66 Mg C ha^{-1} yr^{-1} in 0–10 cm under SoyEx, from 0.85 to 0.96 Mg C ha^{-1} yr^{-1} in 0–20 cm under MaiEx, and from 0.69 to 0.86 Mg C ha^{-1} yr^{-1} in 0–40 cm under CasEx. Considering the cumulative SOC stock, our results revealed that all the NT systems significantly ($P > 0.05$) increased cumulative SOC stock across the soil profile in MaiEx and CasEx. In SoyEx, significant increase in SOC stock was limited to the top 0–20 cm under NT monocropping, whereas NT crop rotation systems had significantly accumulating SOC stock from 0 to 80 cm depths (Table 5) (the cumulative SOC stock in 2011 is presented in Table S9 in the supplementary materials). When considering the whole profile (0–100 cm), the annual SOC accumulation rate under NTs ranged from 0.65 to 1.00, 0.86 to 1.47, and 0.70 to 1.07 Mg C ha^{-1} yr^{-1} in SoyEx, MaiEx and CasEx, respectively.

Consistent with our findings, with the intensive NT systems and high C inputs retained to the soils, other studies reported that long-term NT with the use of cover crops increased SOC stock beyond the surface and the whole soil profile (Diekow et al., 2005; Boddey et al., 2010; Olson et al., 2014). From three long-term experiments (15–26 years) on Ferralsols in South Brazil, no-tillage with intensive cropping systems of maize and soybean production increased SOC with annual accumulation rates between 0.04 and 0.88 Mg ha^{-1} in 0–30 cm, and from 0.48 to 1.53 Mg ha^{-1} yr^{-1} 0–100 cm (Boddey et al., 2010). After 12 years of NT adoption with the use of cover crops for soybean and maize rotation in a humid continental sloping land in Illinois, USA, SOC stock recovered from its initial SOC loss under CT before the experiment implementation, with accumulation rates of 0.42, 0.78, and 1.21 Mg C ha^{-1} yr^{-1} at 0–15, 15–75, and 0–75 cm, respectively (Olson et al., 2014).

SOC storage and stabilization could be explained by several processes: (i) continuous supplies of large quantities and diverse qualities of plant biomass-C inputs to the soil (Sá et al., 2014); (ii) the transformation of this biomass-C by microbial communities into various organic C forms (Frasier et al., 2016; Schmidt et al., 2019); (iii) the stabilization of newly derived C by physical protection, binding with organo-mineral particles, and biochemically stabilization through the formation of recalcitrant soil organic matter (Six et al., 2002); and (iv) distribution of SOC over the soil profile through biological processes, from root systems (Lorenz and Lal, 2005) and soil fauna (Lavelle et al., 2016).

In NT systems, multiple crop species were sown in the same unit area of land through the rotation of cash crops and the use of cover crops by intercropping or during the fallow period, producing a large quantity and diverse quality of C inputs above- but also below-ground that were retained to the soils. In our experiments, the annual biomass C inputs retained in the NT soils ranged from 3.61 to 6.07 Mg ha^{-1} yr^{-1}, versus 1.36 to 2.20 Mg ha^{-1} yr^{-1} under CT (Table 1). In a clayed Oxisol of Brazil, a 16-year-old experiment revealed that NT was more effective than CT at converting biomass-C inputs into SOC, with a C conversion ratio in 0–40 cm depth of 0.35 compared to 0.07 under NT and CT, respectively (Sá et al., 2014). In addition, integration of cover crops into the crop production system led to a significant increase in SOC. From the observation of 139 plots at 37 sites from the tropics and temperate zone and diverse soil types, Poeplau and Don (2015) reported that the use of cover crops led to an average SOC

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

accumulation rate of 0.32 Mg C ha^{-1} yr^{-1} at 22 cm depth. Association of tropical legume cover crops in maize production led to increased SOC stock in the surface as well as the whole soil profile. Diekow et al. (2005) found that the SOC accumulation rate in the legume-based cropping systems was 0.83 and 1.42 Mg C ha^{-1} yr^{-1} in the 0–17.5 cm and 0–107.5 cm layer, respectively after 17 years of NT adoption in a Brazilian Acrisol. From a 30-year-old experiment in a Brazilian Acrisol, legume cover crops were twice as effective in storing C as mineral N fertilization, with 1 kg of residue C input being transformed to 0.15 kg of SOC (Veloso et al., 2018).

During the decomposition process, microbial communities use the rapidly decomposable materials as energy sources, while the recalcitrance and other labile compounds materials act as the glue to bind soil mineral particles together (Witzgall et al., 2021). This process is a pathway for the formation of soil micro-aggregates (Bot and Benites, 2005). The continuous supply of biomass C inputs to the soil associated with microbial decomposition without soil mechanical disturbance creates a favourable environment for the emergence of soil macroaggregates (Crews and Rumsey, 2017). Organic carbon inside soil aggregates is physically protected from microbial oxidation as well as strongly associated with the organo-minerals, leading to SOC stabilization over time (Powlson et al., 1987; Lützow et al., 2006). In the same experiments as in our study but after 3 years of NT adoption, Hok et al. (2021) reported that soil aggregation was one of the main stabilization mechanisms, providing physical protection to the newly derived C into the soil microaggregates protected by macroaggregates. From our knowledge of the literature, the high SOC accumulation rate recorded under cassava-based NT cropping systems is relatively unique and, in addition to the residues of cover crops and maize under the biannual cropping system, the nature of the cassava residues that was retained into the field with high cellulose and lignin

https://doi.org/10.5194/egusphere-2024-541
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contents (Veiga et al., 2016) may explain this result. Considering the challenges faced by smallholder farmers in Cambodia with low financial resources and/or high level of indebtedness, the main strategy should focus on enhancing nutrients cycling through continuous biomass-C inputs under no-till cropping systems plus a combination of actions to reduce nutrient removal from cassava fields through the non-removal of leaves and of a proportion of stalks, that may also help to reduce the impact of nutrients deficiency. In addition, the tolerance of cassava to acidic soil, its ability to grow on depleted and degraded soils related to the occurrence and synergistic effects of arbuscular mycorrhizal fungi (Howeler et al., 1982; Howeler and Sieverding, 1983), and plant growth-promoting rhizobacteria (PGPR) (Balota et al., 1999), its nutrient recycling ability through leaf litter and when the stalks are not used as planting materials and kept into the field, could be used to advance in soil and cropping system sustainability (Fermont et al., 2008). This possible use by farmers of cassava cropping systems as a strategy for regenerating soil fertility was also emphasized by Saïdou et al., (2004) and Adjei-Nsiah et al., (2007) in Benin and Ghana, respectively. Long-term NT adoption has been shown to significantly improve soil structure, soil porosity and pore connectivity (Cooper et al., 2021) contributing to the improvement of water infiltration, gas exchanges and microbial activities, and roots development to deeper soil profile (Rosolem et al., 2016). In addition, aerobic condition of soil aggregates would enhance SOC stability in unsaturated soils (Zhang et al., 2021). Sisti et al. (2004) showed that increased C accumulation in NT soil below 30 cm depth could be explained by greater root density when compared with CT. Another possibility is that organic residues from upper layers were transported downward by soil meso- and macro-fauna organisms, which could have been favoured by better environmental conditions provided by the continuous C flow and soil structure enhancement under NT systems (Lavelle et al., 2016).

Table 5. Cumulative SOC stock in 2021 and cumulative SOC stock change 2021-2011.

Experiments[a]	**Approximate soil depth (cm)**	**Cropping systems**[b]							
		CT	**NT1**	**NT2**	**NT3**	**CT**	**NT1**	**NT2**	**NT3**
		Cumulative SOC stock 2021 (Mg C ha^{-1})				**Cumulative SOC stock change 2021-11 (Mg C ha^{-1})**			
MaiEx	0-10	19.98 Ab	25.93 Aa	25.18 Aa	24.40 Aa	**1.24**	**8.30**	**7.06**	**6.64**
	0-20	38.26 A	42.24 A	42.26 A	40.72 A	**3.53**	**9.37**	**9.56**	**8.49**
	0-40	58.16 A	60.85 A	62.55 A	59.37 A	**4.61**	**10.94**	**14.03**	**9.22**
	0-60	70.74	73.03 A	75.29 A	71.68 A	4.19	**11.76**	**15.07**	**9.40**
	0-80	79.75	82.61 A	84.93 A	81.01 A	3.01	**12.45**	**14.89**	**9.21**
	0-100	86.92	90.13 A	92.90 A	88.27 A	1.75	**12.97**	**14.71**	**8.59**
SoyEx	0-10	19.41b	22.91 Aab	24.19 Aa	24.38 Aa	0.46	**4.00**	**4.96**	**5.61**
	0-20	37.76	39.70 A	41.46 A	41.81 A	1.52	**3.76**	**6.61**	**6.37**
	0-40	60.10	62.35	60.72 A	61.50 A	3.45	5.43	**8.55**	**8.19**
	0-60	75.30	78.21	73.72 A	74.59 A	4.82	6.46	**9.81**	**9.88**
	0-80	86.45	90.16	83.20 A	83.90 A	5.22	7.03	**9.96**	**10.24**
	0-100	95.22	99.53	90.23	91.15	4.78	6.53	9.18	10.03
CasEx	0-10	17.41 Ac	19.00 Abc	20.50 Aab	21.43 Aab	**1.46**	**3.57**	**3.09**	**3.86**
	0-20	34.56 Ab	35.66 Aab	37.13 Aab	37.66 Aa	**2.39**	**6.04**	**3.98**	**5.69**
	0-40	52.74	54.50 A	57.90 A	56.93 A	1.45	**8.28**	**6.88**	**8.57**
	0-60	65.86	67.93 A	71.84 A	69.92 A	1.00	**9.72**	**8.05**	**9.82**
	0-80	76.97	78.71 A	82.46 A	80.10 A	1.20	**10.58**	**7.93**	**10.25**
	0-100	86.44	87.42 A	91.07 A	88.01 A	1.35	**10.68**	**6.97**	**9.46**

[a] MaiEx: Maize-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment.

[b] CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till associated with cropping systems as described in Table 1. Uppercase letters accompanying the values indicate significant difference within the same treatment between 2011 and 2021 (diachronic) at the same soil depth at $P \leq 0.05$ (Tukey's test). Values of cumulative SOC stock change in bold indicate changes for a given treatment and depth between 2021 and 2011 are significantly different from 0 at $P \leq 0.05$ (Tukey's test).

https://doi.org/10.5194/egusphere-2024-541
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In our study, the SOC stock in the whole soil profile (0–100 cm) under CT and for the three experiments remained constant, which could be attributed to the fully retained crop residues (i.e., mungbean, rice, and maize) in MaiEx and (i.e., sesame and soybean) in SoyEx and partially retained cassava's fallen leaves and stalks in CasEx (Table 1), indicating that it reached a new SOC equilibrium stage. The high clay content has also contributed to the stabilisation of the MAOC that accounted, in 2021, for 97.2% of the carbon concentrations along the soil profile.

Under a synchronic approach, considering CT as the reference, the SOC stock change rates in 2021 under NT systems ranged from 0.13 to 0.60, -0.50 to 0.43, and 0.10 to 0.46 Mg C ha^{-1} yr^{-1} in MaiEx, SoyEx, and CasEx, respectively (Fig. 6). When compared with the diachronic approach, this corresponds to an underestimation of 146 to 536%, 51 to 347%, and 51 to 997% in MaiEx, SoyEx, and CasEx, respectively (Table 3). In Brazil, from on-farm assessments of the SOC dynamics under long-term NT systems in tropical heavy clayed soils Neto et (al., 2010) and Junior et al., (2013) reported that synchronic approach led to the bias in the annual SOC accumulation rates under NT systems when compared with diachronic approach. The main factors associated with the errors could be the underlying heterogeneities of the soil conditions prior to the conversion to NT systems that are hard to capture despite all the steps of the methodologically precautious measurements being implemented properly (Neto et al., 2010; Junior et al., 2013). Our findings clearly emphasize the importance of the diachronic approach in accurately estimating the effects of long-term CA and NT systems on SOC storage, as well as providing a proper interpretation of their roles in climate change mitigation through SOC sequestration.

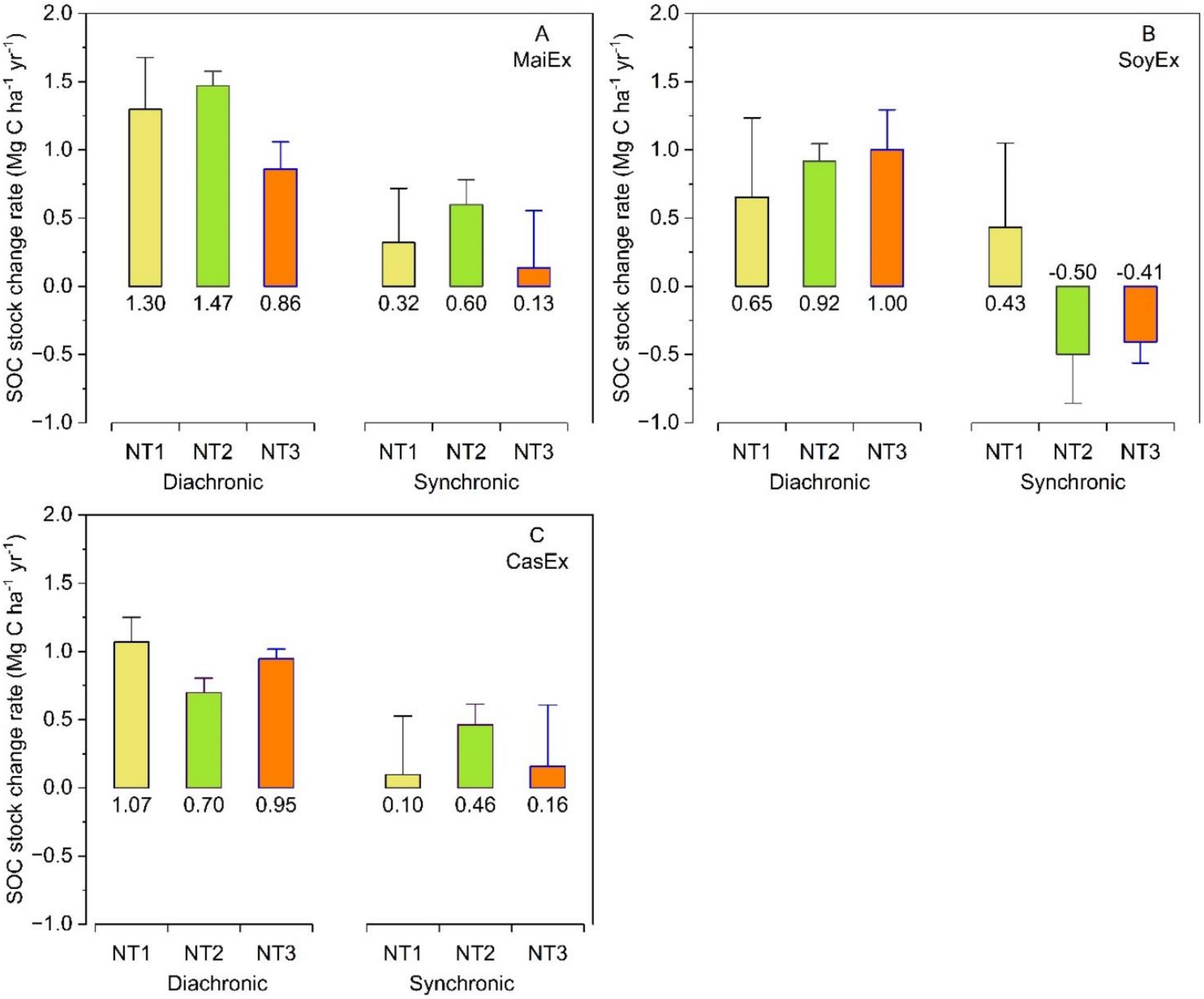

Figure 6. Comparison between the diachronic and synchronic approaches to estimate SOC stock change rate (0–100 cm) from 2011 to 2021 under NT systems in the tropical red Oxisol of Cambodia by considering SOC stock under CT as the control for the stock change rates of NT systems in 2021 for the calculation in the synchronic approach (n = 3; error bars = SE).

4.2 Change in N stock

In addition to increasing SOC stock in the surface and the whole soil profile, Diekow et al. (2005) found that soil total N stock was significantly increased by an average of 27% at the surface (0–17.5 cm) and by 6% in the whole profile (0–107.5 cm) after 17 years of NT maize with the use of tropical legume cover crops and N fertilization in comparison to its origin state under native grassland as a reference in a Brazilian Acrisol. Sá et al., (2014) reported a strong positive relationship ($R^2 = 0.89$, $P < 0.0002$) between the soil N and SOC stock accumulation; each unit of N stock accumulation contributed to the sequestration of 10.2 Mg C ha^{-1} at the top 0–10 cm under long-term (16-year) continuous NT maize-based production of Brazilian's Oxisol.

However, the diachronic assessment in our study showed that soil N stock under NT systems significantly increased only in the topsoil (0−5 cm) in MaiEx and SoyEx, while the stock remained stable in CasEx. The significant decline of N stock under NT systems, although with variability across NT systems and experiments, was detected below 20 cm. When considering the whole profile (0–100 cm), significant depletion of N stock was observed under the NT monocropping systems, with a loss rate at -0.10 and -0.17 Mg N ha^{-1} yr^{-1} in SoyEx (NT1-Sb) and CasEx (NT1-Cs), respectively. Under NT crop rotation systems, despite non-significant, N stock tended to decrease across the three experiments, with a depletion rate ranging from -0.03 to -0.09 Mg N ha^{-1} yr^{-1} (Table 4).

The depletion of N stock under NT was reported from short- (Wuaden et al., 2020) to longer-term NT adoption (Delgado, 2023). From a short-term (5-year) conversion of native grassland to cropland under NT adoption with a double cropping with maize as a cash crop followed by black oat as a cover crop in Brazil's Rhodic Nitosol, NT soils had significant losses of soil total N in comparison with the original stocks under grassland throughout the soil profile, with the exception of the 0−5 and 10−20 cm soil layers. Considering the whole profile (0–60 cm), soil total N was depleted by 1.7 Mg N ha^{-1}, equivalent to an annual loss rate of -0.34 Mg N ha^{-1} yr^{-1} after 5 years of grassland conversion to NT (Wuaden et al., 2020). Results from a 12-year experiment in the US (0-120 cm depth) in an irrigated NT continuous maize rotation where mineral N were applied at different rates indicated that even NT could potentially have significant net N loss with an average loss of −15 kg N ha^{-1} yr^{-1} at the top 30 cm of soil regardless of N application rate (Delgado, 2023).

It is a rather surprising finding to observe an increase in SOC and a simultaneous soil N depletion. Associating legume cover crops in the cropping system did not enhance soil N through biological N fixation (Rosolem et al., 2016). In general, N is an important factor contributing to SOC storage (De Vries, 2014; Kirkby et al., 2014). Nutrient reserves are among other factors that determine soil C storage capacity (Lal, 2018). Therefore, more studies on nutrient availability and their stoichiometry relationship including in deeper layers (>100 cm), on the N use efficiency and N cycling are needed to understand the driving mechanisms of the N dynamics under these NT systems.

To date, few studies on the impact of no-till systems on N gains or N losses, N use efficiency and changes in soil N have been conducted, and the results vary depending on the period of NT adoption and sampling depths (Congreves et al., 2017; Delgado, 2023). Further research is needed to understand the driving mechanism of the N dynamics under NT systems by considering deeper layers (>100 cm) for making informed decisions regarding sustainable soil fertility management and crop production systems. Positive accumulation rates of SOC stock, recorded under NT systems, could not be sustained on the long-term as the depletion of the N stock may lead to nutrient scarcity of other nutrients (P, S, Ca^{2+} and Mg^{2+}) that is the driving force limiting SOC accumulation. Further analysis is needed to assess the coming changes in SOC and N stocks along with the content of nutrients and its potential impact on the rate of SOC accumulation or depletion (Kirkby et al., 2013).

4.3 SOC and N stocks recovery after land use change

Conversion of native vegetation to cropland under conventional plough-based tillage depletes SOC (Sisti et al., 2004; Sá et al., 2015; Wuaden et al., 2020) due to soil structure disruption by the mechanical disturbance, low C inputs, and accelerates the SOC mineralisation rate by exposing SOC that was encapsulated inside soil aggregates to microbial oxidation (Balesdent et al., 2000). Even if this comparison with RV was restricted to 0-20 cm depth, the present study showed that NT systems can restore SOC stock that was lost during crop production under conventional tillage after the conversion of native vegetation to cropland and before the experiments' establishment. At 0–20 cm depth, the annual SOC accumulation rates of NT systems ranged from 0.85 to 0.96, 0.38 to 0.67, and 0.40 to 0.61 Mg C ha^{-1} yr^{-1} under MaiEx, SoyEx and CasEx, respectively. This could be attributed to the long-term NT systems adoption with multiple crop species through cash crop rotation and cover crop association producing high and diverse biomass-C inputs retained in the soil, leading to an increase in SOC stock across the whole profile. After 12 years of experimentation, Neto et al., (2010) found that SOC stocks under NT mulch-based management systems were no longer significantly different from the stocks of natural Cerrado vegetation in Brazil. Sá et al., (2014) reported the recovery of SOC stock under NT to the reference vegetation an accumulation rate of 0.84 Mg C ha^{-1} yr^{-1} at 0–20 cm after 16 years of continuous NT with an average annual biomass C input of 4.40 Mg ha^{-1} yr^{-1}.

Noticeably, SOC stock under CT was significantly lower than RV in 2011 in all the three experiments, but SOC stock did not differ from RV in SoyEx and MaiEx, while the stock remained stable in CasEx in 2021 (Fig. 3). Because the SOC stock under CT soils have been depleted over the 70 years after natural forest conversion to cropland (see the history of land use change in Hok et al., 2015) they represent a potential C sink. Therefore, the gain of SOC stock under CT could be explained by the annual full retention of crop residues in MaiEx and SoyEx over the experiment period (Table 1) along with the high clay content of this oxisol.

The recovery on N stock to RV under the NT systems in MaiEx and as well as all the tillage systems (including CT-Sb) in SoyEx could be explained by the association of legume crops and the use of mineral fertilizer as described in Table 1 and 2.

4.4 Carbon and N in size fractions and stabilization processes in NT systems

Particulate organic matter (POM) and mineral-associated organic matter (MAOM) are the two main fractions of the SOC pools. They differ in physical and chemical characteristics as well as their turnover rates. POM is more sensitive to soil tillage and land use than MAOM and total SOC (Blanco-Moure et al., 2013; Kan et al., 2021). At 0–10 cm, NT systems significantly increased C-POM by 115%, 118%, in MaiEx and SoyEx, respectively, and by 37% in CasEx although this was not significant. NT systems also significantly enhanced C-MAOM by 33%, 21%, at the 0–10 cm depth in MaiEx and SoyEx and even deeper to the soil profile > 20 cm in CasEx (Fig. 4). These increases could be attributed to the continuous supply of large amounts and diverse biomass-C inputs to the soil surface, through the diversity of the root systems along with the low level of soil disturbance under NT systems (Sá et al., 2014; Briedis et al., 2018). From an incubation of labelled litter, Witzgall et al., (2021) found that the occlusion of organic matter into aggregates and the formation of organo-mineral associations occurs concurrently on fresh litter surfaces regardless of soil structure. In addition, the increase in C-MAOM is attributed to C transfer from POM and other labile C pools. Over time, these compounds are transferred to more stable pools,

https://doi.org/10.5194/egusphere-2024-541
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creating associations with mineral colloids, with MAOM being more stabilized (Briedis et al., 2018). Rosolem et al. (2016) conducted 3-year successive experiments to assess the above- and belowground effects of a wide range of tropical grasses and legume cover crops, which were the same species that were used under the NT systems in our experiments, in combination with no-till soybean-based cropping systems in Brazilian tropical clayed Rhodic Ferralsol on total organic C and N stocks and in POM fraction. They reported that the presence of C4 deep-root grass cover crops during the fallow period significantly increased total organic C and POM. Furthermore, legume cover crops contributed to maintaining the C/N ratio in the topsoil layers, which could keep increasing C over time. Beside the aboveground biomass, root systems of cover crops are also an important C inputs for SOC accumulation due to their capacity to grow deeper in the soil profile, during the dry season for some, exploring large volume of soil (Rosolem et al., 2016; Sokol et al., 2019), releasing large quantities of roots exudates, and recycling nutrients (Rosolem et al., 2005). The increase in C-POM and C-MAOM shows that NT systems with the use of cover crops is a key strategy to promote both SOC storage and long-term SOC stabilization.

In contrast to C-POM and C-MAOM, our results showed that NT cropping systems only increased the amount of N-POM at the uppermost soil surface (0–10 cm) in MaiEx and SoyEx over the past 10 years. However, significant decreases in N-POM and N-MAOM were observed below 40 cm and 5 cm, respectively (Fig. 5).

5 Conclusion

The present study showed that, over 10 years, variable effects were observed among the three NT systems and in the three experiments. Considering layer by layer, the significant effect of NT systems on SOC

https://doi.org/10.5194/egusphere-2024-541
Preprint. Discussion started: 7 March 2024

was observed in the topsoil 0–10, 0–20 and 0–40 cm in SoyEx, MaiEx, and CasEx, respectively. When considering the cumulative layers, all the NT systems significantly increased SOC stock across the soil profile under MaiEx and CasEx. For SoyEx, the cumulative SOC stock is restricted to 0–20 cm under NT monocropping and to 0–80 cm under NT crop rotation systems. In the whole profile (0–100 cm), the annual SOC cumulative rate in NT systems ranged from 0.86–1.47, 0.65–1.00, and 0.70–1.07 Mg C ha^{-1} yr^{-1} in MaiEx, SoyEx, and CasEx, respectively. The main impact of NT systems on C-POM and C-MAOM was observed in the top 0–10 cm in MaiEx and SoyEx, whereas significant effect on C-MAOM in soils under NT systems was found from 0–40 cm in CasEx.

In contrast to SOC, N concentration and stock in NT soils only increased in the surface layer (0–5 cm). Although an increase of N-POM under NT systems was found in the top soils, a decrease was observed in the subsurface layers. Surprisingly, intensive NT systems caused the depletion of N-MAOM with significant losses observed below 5, 20, and 40 cm in CasEx, MaiEx and SoyEx, respectively. This resulted in significant N stock depletion below 40 cm and in the whole profile, particularly, under soybean and cassava NT monocropping systems.

Overall, our findings reveal that diachronic sampling is crucial for proper measurements of the impacts of NT systems on SOC dynamics with time. Adopting NT cropping systems accompanied by diversified crop species and high biomass C inputs with an absence of heavy soil disturbance in the long-term significantly increased SOC stock and pools in the tropical red Oxisol of Cambodia. The study highlights the potential of NT cropping systems for SOC accumulation and stabilization over time, even for cassava, which is known to have severe environmental impacts and soil degradation, but raises questions about soil N dynamics. Further research on the N use efficiency, N cycles, and nutrient availability and their stoichiometry relationship by considering deeper layers (> 100 cm) is needed to understand the mechanism driving N loss in NT systems for making informed decisions regarding sustainable soil fertility management and crop production systems.

6 Code and data availability

All data are freely available on the CIRAD data repository https://doi.org/10.18167/DVN1/NNBBAQ (Leng et al., 2024).

7 Author contributions

VL co-established and managed the experiments, carried out the fieldworks, managed all sample collection campaigns and laboratory works, and wrote the first manuscript draft. FT co-managed the experiments, provided support to the team for the last 8 years and raised funds along with GDA/DALRM to sustain these long-term experiments. LT, RC, and FT conceptualized the research, supervised the field operations, analytical procedure, data computation, and reviewed the manuscript. VS, PL, LH, JS and CB gave advices for the analytical procedures, data calculation and manuscript improvement. PM significantly contributed to the implementation of the field operations, sample collection and lab works. TF performed the statistical analysis. SB raised funds, designed, established and managed the experiments during the first years and contributed to the manuscript improvement.

8 Competing interests

One co-author is a member of the editorial board of SOIL.

9 Acknowledgements

This research has been fully funded by the Agroecology and Safe Food System Transitions in Southeast Asia (ASSET) project. The project is co-financed by French Development Agency (AFD), European Union (EU), and French Facility for Global Environment (FFEM) under the conventions CZZ2453 and CZZ2868. We thank the DALRM/GDA/MAFF for providing access to the experiments and supporting the paperwork authorization for sample shipments to Switzerland. We further acknowledge CIRAD, France, for its strong collaboration with GDA and for continuing to provide the technical support for maintaining these long-term experiments. Additional thanks to CARDEC's team, who manages the field operations. Special appreciation to Britta Jahn-Humphrey of the Sustainable Agroecosystem, ETH Zurich, for processing paperwork and overseeing the measurement of all the SOC and N.

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