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1	Diachronic assessment			
2	of soil organic C and N dynamics under long-term no-till		Formatted: Font color: Auto	
3	cropping systems in the tropical upland of Cambodia		Formatted: Font color: Auto	
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4	Vira Leng ^{1,2*} , Rémi Cardinael ^{3,4,5} , Florent Tivet ^{1,3,6} , Vang Seng ¹ , Phearum Mark ¹ ,		Formatted: Font color: Auto]
5	Pascal Lienbard ^{3,7,8} Titouan Filloux ³ Johan Six ⁹ Lyda Hok ¹⁰ Stéphane Boulakia ³		Formatted: Font color: Auto	
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6	Clever Briedis ¹¹ , João Carlos de Moraes Sá ¹² , Laurent Thuriès ^{13,14}		Formatted: Font color: Auto	
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30 Abstract

31	No-till (NT) cropping systems have been proposed as a strategy to combat soil degradation by storing soil
32	organic carbon (SOC) and total nitrogen (TN). No till (NT) cropping systems have been proposed as a
33	potential strategy to combat soil degradation and global warming by storing soil organic carbon (SOC)
34	and nitrogen (N). Yet, there are ongoing debates about the real benefits of NT systems and factors
35	influencing SOC and N accumulation. Assessing the dynamics of SOC and N on the long term is needed
36	to fill knowledge gaps and provide robust scientific evidence for potential additional SOC storage. We
37	quantified the impacts of NT cropping systems on the changes in SOC and -TN stocks in bulk soil and in
38	particulate and mineral-associated organic matter fractions (POM and MAOM)-stocks and fractions,
39	down to 100 cm depth, from three 13-year-old experiments in a tropical red Oxisol in Cambodia using
40	diachronic and equivalent soil mass approaches., comparing conventional tillage (CT) to NT
41	monocropping and NT crop rotation systems using a diachronic and equivalent soil mass (ESM)
42	approach. Established in 2009 and arranged The three experiments in a randomized complete block design
43	with triplicates, the experiments included comprised maize (MaiEx)-, soybean (SoyEx)-, and cassava
44	(CasEx)-based cropping systems experiments trials, hereafter called MaiEx, SoyEx, and CasEx,
45	respectively Each experiment comprised three treatments: (1) monocropping of main crops (maize,
46	soybean, and cassava) under conventional tillage (CTM); (2) comparing with-monocropping of main
47	crops under NT systems with the use of cover crops (NTM); and (3) bi-annual rotation of main crops
48	under NT systems with the use of cover crops (NTR), both crops being presented every year and
49	represented by NTR1 and NTR2. Soil samples were collected in 2021, 10 years after the
50	first-last sampling (in 2011), at 7 depths: 0 5, 5 10, 10 20, 20 40, 40 60, 60 80, and 80 100 cm. Over

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51	the 10 year period (2011 2021), significant impacts on SOC stock and its vertical distribution differed
52	among the NT systems and in the three experiments <u>All the NT systems significantly ($p < 0.05$) increased</u>
53	SOC stock in the topsoil of the stock in SoyEx and MaiEx and down to 40 cm in CasEx. C. In MaiEx and
54	CasEx, the soils under all the NT systems significantly ($P > 0.05$) accumulated SOC stock across the soil
55	depths, with the accumulation ranging from 6.97 to 14.71 Mg C ha ⁴ in the whole profile (0-100 cm). In
56	SoyEx, significant increase in SOC stock was limited to the top 0-20 cm under NT monocropping,
57	whereas NT crop rotation systems had significantly accumulating SOC stock from 0 to 80 cm depths.
58	When considering the whole profile (0-100 cm)-(0-100 cm), the as a single stratum, the annual-SOC
59	stock cumulative rate in NT systemsSOC-accumulation rates rangeded from 0.86-1.47, 0.65-1.00, and
60	0.70–1.07 Mg C ha ⁻¹ yr ⁻¹ in MaiEx, SoyEx, and CasEx, respectively. In the top 0–10 cm, NT systems
61	significantly increased C concentration in particulate organic matter (POM) by 115%, 118%, in MaiEx
62	and SoyEx, respectively, and by 37% in CasEx aAlthough SOC stock significantly increased in CTM in
63	0-20 cm in MaiEx and CasEx, it remained stable in 0-100 cm in all the experiments. not significantly
64	Similarly, at 0-10 cm depth, NT systems significantly enhanced C concentration in the mineral-associated
65	organic matter (MAOM) by 33%, 21%, in MaiEx and SoyEx, respectively. Significant increase of C in
66	MAOM was also observed from 0 to 40 cm in CasEx. In 0-5 cm, In contrast, total N stock in NTR
67	systemoils cropping systems significantly increased TN stock in all the experiments, in the surface 0-5
68	em depth but while in NTM system it was only significant in MaiEx and SoyExdecreased below 10 cm
69	and in the whole profile. In 0-100 cm, TN stock in all the experiments remained stable under NTR
70	systems, whereas significant decrease was observed under NTM systems in SoyEx and CasEx. Although
71	C(0-100 cm), particularly under The significant effects of NT monocropping with an annual loss rate of

72	0.10 and 0.17 Mg N ha ⁺ yr ⁺ in SoyEx and CasEx, respectively. Although NT <u>A significant increase</u>
73	systems increased N cioncentrationSO in _POM stock significantly increased under all NT systems
74	limited to 0-in the top 0-10 cm of MaiEx and SoyEx, a decreasing trend was observed below 10 cm
75	depth. The N concentration in POM under NT systems in CasEx also decreased with soil depth. From
76	2011 to 2021, N concentration in MAOM was foundunder NT systems remained stable in MaiEx and
77	SoyEx in the top 0–105 cm in NT cropping systems, but significant decreases in MaiEx and CasEx-SoyEx,
78	theall the NT systems significantly increased in SO C-MAOM stock in the 0-10 cm layer in MaiEx and
79	SoyEx and was found stock down to 40 below 5 cm was detected in CasEx. All the In contrast to C pools,
80	NT systems Tfractionssignificantly increased N-POM stock in 0-10 cm in MaiEx and SoyEx, while a
81	significant decreased in N-MAOM stock was observed below 5 cm in CasEx and below 40 cm in MaiEx
82	and SoyEx.
83	Our findings suggesthowed that adopting-long-term_NT cropping-systems with-diverse crop and cover
84	erop-species_diversification and high biomass C inputs in the long term leads to SOC accumulated
85	SOCion not only ion the surface but also in dtheeper whole profile layers, by increasing both the SOC
86	pools in the POM and MAOM-size fractions, even on in the cassava-based system, which is believed to
87	be an annual crop that could cause serious soil fertility depletion. This study highlights the potential of
88	NT-eropping systems ftor storinge SOC over time, but raises questions about soil N dynamics, but raises

89 questions about soil N dynamics.

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Land and soil degradation is a global challenge with consequences not only for food and nutrition security 91 92 but also for livelihoods, environmental pollution, climate change, water scarcity, and biodiversity. The 93 main processes that cause soil degradation are water and wind erosion, chemical depletion, physical 94 deterioration, decline in soil organic carbon (SOC)-pools, loss in biodiversity, acidification, and salinity 95 (Lal, 2015a; Stavi and Lal, 2015; Dragović and Vulević, 2020; Barbier and Di Falco, 2021). It was 96 estimated that about 562 million ha of land are degraded worldwide, with 5 to 10 million ha of land lost 97 each year as a result of severe degradation (Stavi and Lal, 2015; Nkonya et al., 2016). The major factors 98 contributing to soil degradation are deforestation and land clearance, the overuse of agrochemicals, and 99 inappropriatensive agricultural management practices (Dragović and Vulević, 2020). Tropical soils have 100 the highest risks of degradation due to the combination of high rainfall intensity and the ongoing 101 intensification of agriculture to meet the food demand of a fast-growing population, which is also 102 constrained by the limited availability of land to be converted to cropland Barbier and Hochard, 2018; 103 Craswell and Lefroy, 2001; Barbier and Di Falco, 2021). 104 Cambodia, located in the tropical region of Southeast Asia, is one of the highest land degradation hotspots 105 in the world, and about 6055% of the country's population reside in these hotspot areas (UNCCD, 2018). 106 In the last two decades, human-induced activities including deforestation, land clearance for agriculture, 107 climate change, and inappropriatensive 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

109 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

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111	the Northwest rainfed uplands, like in other parts of the country, studies on soil erosion at field scale and
112	modelling reported that the annual soil loss rate in conventional plough-based tillage (CT) ranged from
113	0.33 to more than 80 Mg soil ha ⁻¹ yr ⁻¹ , depending on soil type and land slope (CARDI, 2017; Nut et al.,
114	2021; Sourn et al., 2022). The amplitude of soil erosion increased by 41% from an annual erosion rate of
115	2.92 Mg soil ha ⁻¹ yr ⁻¹ in 1998 at the beginning of the forest conversion to agriculture with extensive, more
116	diversified farming practices to 4.98 Mg soil ha ⁻¹ yr ⁻¹ in 2018 under CT maize_ and cassava-based
117	monocropping systems (Nut et al., 2021; Sourn et al., 2022, 2023). It was estimated that approximately
118	3-4 mm of topsoil is washed away annually for this Northwestern region of Cambodia (Nut et al., 2021;
119	Sourn et al., 2023). Erosion induces soil degradation and <u>a SOC-loss of SOC for the eroded fields</u>
120	(Polyakov and Lal, 2004). It was estimated that from 2000 to 2010, Cambodia lost approximately 1.98
121	million Mg C in the top 0–30 cm depth as the consequence of forest conversion to other land uses (MAFF,
122	2018). Cambodian soils are seriously threatened by inappropriate intensive agricultural systems. The
123	returns on taking actions against land degradation through restoration and adoption of sustainable
124	agricultural management practices are estimated at 3 US dollars for every dollar invested in restoring
125	degraded land in Cambodia, highlighting the strong economic benefits (UNCCD, 2018). Therefore, taking
126	actions to reverse the trend of soil degradation through restoration and adopting sustainable agricultural
127	management practices highlights the strong economic benefits while combating soil degradation in the
128	country (UNCCD, 2018).
129	SOC serves as the foundation of soil physical, chemical, and biological processes that sustain essential
130	ecosystem functions, and it is the reservoir of plant nutrients and energy for biota (Lal. 2015a). Therefore,

adopting sustainable management practices that lead to increase in SOC content_stock (Beillouin et al.,

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132	2023) is part of the key strategies to reverse the soil degradation trends and to minimize the economics
133	and environmental impacts related to land degradation (Lal, 2015a; Obalum et al., 2017). Studies reported
134	that agricultural practices, in particularly those based on CT, weaken soil structure, accelerate soil erosion,
135	and deplete SOC stock (Tivet et al., 2013; Sá et al., 2014; Briedis et al., 2018; Oliveira et al., 2020; Tiecher
136	et al., 2020). By contrast, conservation agriculture (CA), defined by three key principles, (i) minimum or
137	no soil disturbance, (ii) permanent soil cover with mulch or cover crops, and (iii) crop diversification
138	through rotation or association, is a potential strategy to overcome soil degradation (Luo et al., 2010; Lal,
139	2015b; Powlson et al., 2016; Obalum et al., 2017). No-till cropping systems (NT), are part of the
140	conservation agriculture practice approach, and involve a range of practices with a reduction or an absence
141	in soil tillage and a high diversity of plant crop and cover crop species, amount and frequency of biomass-
142	C inputs (e.g., main crops and cover crops). The benefits of CA and NT cropping systems on soil health
143	improvement have been reported worldwide. Since 2004, the conservation agriculture research for
144	development programs have been initiated by the joint collaboration between the General Directorate of
145	Agriculture (GDA) and Centre de Coopération Internationale en Recherche Agronomique pour le
146	Développement (CIRAD), France. Diversified NT cropping systems enhance both the SOC stock in the
147	topsoil surface layer only after a several years of implementation (Hok et al., 2015) and pools (Sá et al.,
148	2014; Briedis et al., 2018; Sithole et al., 2019; Cooper et al., 2021; Rodrigues et al., 2022), especially
149	through an increase in physical protection of SOC particulate organic C (Sithole et al., 2019) and mineral-
150	associated organic C inside soil aggregates (Six et al., 2002; Sithole et al., 2019; Rodrigues et al., 2022)
151	inside soil aggregates. In addition, numerous studies have reported the co-benefits of NT cropping
152	systems on soil health enhancement (Pheap et al., 2019; Koun et al., 2023), increased water infiltration,

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153	reduced soil erosion (TerAvest et al., 2015; Sithole et al., 2019), and enhanced microbial activities (Hok
154	et al., 2018) and abundance (Lienhard et al., 2013). Yet, there are still arguments about the benefits of CA
155	and-NT cropping systems and associated factors that determine SOC accumulation. Particulate organic
156	matter (POM) and mineral-associated organic matter (MAOM) are the two main fractions of the SOC
157	pools. They differ in physical and chemical characteristics as well as their turnover rates. POM is more
158	sensitive to soil tillage and land use than MAOM and total SOC (Blanco-Moure et al., 2013; Kan et al.,
159	2021). In addi <u>Thereforetion</u> , documentation of SOC fractions is desirable for a better understanding of
160	SOC dynamics and stabilization processes (Lavallee et al., 2020). In a meta-analysis with the majority of
161	the studies collecting samples between 0.15 and 0.3 m depth, Powlson et al., (2016) reported that SOC
162	accumulation rate under CA systems ranged from 0.16 to 0.49 Mg C ha ⁻¹ yr ⁻¹ in tropical soils in the Indo-
163	Gangetic Plains and from 0.28 to 0.96 Mg C ha ⁻¹ yr ⁻¹ in Sub-Saharan Africa. In a Ferralsol in Zimbabwe,
164	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
165	under CA, but not change under NT only. However, in meta-analyses, Angers and Eriksen-Hamel (2008)
166	and Luo et al., (2010) found that conversion from CT to NT only changed the SOC distribution in the soil
167	profile but did not significantly increase SOC stock in the whole profile. Boddey et al., (2010) and Xiao
168	et al., (2020) reported that NT significantly increased SOC stock only at the soil surface but not in the
169	deeper layers. It is therefore crucial to quantify SOC change in subsoil when assessing the impact of
170	practices, especially in CA and NT systems.
171	Soil organie OC sequestrationstorage is closely related to soil aggregate structure (Six et al., 2004; Liu et
172	al., 2021). The complexity of cropping systems, characterized by crop species diversity through the use

173 of cover crops, crop rotation, and intercropping, was reported to enhance soil aggregation stability and

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174	the proportion of soil macroaggregates, along with thean increase of SOC (Tiemann et al., 2015; Li et
175	al., 2024). The diversity of crop species increased the quantity and chemical diversity of plant-derived
176	litter inputs, which are the main sources of energy for soil microorganisms, and increased microbial
177	activity and the abundance of fungal and bacterial communities (Tiemann et al., 2015; Zhang et al., 2023).
178	The overall increase in fungal hyphae, plant roots, and aboveground biomass inputs under crop
179	diversification are important organic binding agents that promote the formation of macroaggregates and
180	facilitate the soil aggregation process (Tiemann et al., 2015). Furthermore, the increased amount and
181	diversity of plant-derived C inputs in the forms of crop residues and root exudates provided a suitable
182	microenvironment for soil microorganisms, which promoted microbial growth and turnover (Morugán-
183	Coronado, 2022). The faster microbial growth and turnover rates increased the amount of microbial
184	biomass and necromass, thus increasing SOC (Liang et al., 2011; Prommer et al., 2019). The amount,
185	quality and frequency of the crop residues added to soil under a range of climate-driven decomposition
186	rates, soil mineralogy and profile characteristics are important factors to consider to increase SOC stocks
187	Paustian et al., 1997; Six et al., 2002; Bayer et al., 2006; Ogle et al., 2012; Virto et al., 2012). It has been
188	suggested that the amount of biomass-C inputs was the main factor explaining the variability in SOC
189	storage between sites under NT (Virto et al., 2012). In a synthesis from tropical soils, Fujisaki et al.,
190	(2018) reported that the amount of biomass-C inputs was the main factor driving a positive C budget,
191	enhancing C and N transformations, flow, and SOC stock change. In a meta-analysis in Sub-Saharan
192	Africa, Corbeels et al., (2019) found that not disturbing the soil no-tillage alone does not lead to an increase
193	in SOC stock, but CA systems combining the three principles could. It therefore seems that there is a
194	hierarchy in CA principles to increase SOC stock, the most important one being the permanent soil cover,

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195	followed by a reduction in soil tillage and improved rotations (Shumba et al., 2024). This has been
196	confirmed in a recent second-order meta-analysis where crop residue retention and cover crops were the
197	most efficient CA practices to increase SOC (Beillouin et al., 2023).
198	In addition, uUsing improper methods could mislead the assessment of the long term impact of
199	management practices on the SOC stock. There are tTwo different soil sampling approaches are
200	commonly used for assessing SOC accumulation rates:stock change, the diachronic and the synchronic
201	approaches (Bernoux et al., 2005). The diachronic approach refers to collecting samples on the same field
202	plots over time. The synchronic approach, also known as the space-for-time method, on the other hand,
203	refers to sample collection at the same time from different (often adjacent) field plots under different land-
204	use or management systems (Bernoux et al., 2005; Neto et al., 2010). Neto et al., (2010) and Junior et al.,
205	(2013) revealed that the synchronic approach led to overestimated biassed estimation of SOC
206	accumulation from long-term experiments in Brazil due to spatial heterogeneity and initial land use
207	history. They highlighted that diachronic soil sampling should be used for assessing soil SOC storage
208	rates due to changes in land-use or management patterns because it offers a more comprehensive view of
209	how SOC and N levels change under long-term tillage and cropping systems over time in which non-
210	identical initial soil conditions cannot practically be excluded, making it more accurate and realistic for
211	the investigation of SOC and N dynamics, despite the fact that they are costly and require significant time
212	and resources (Bernoux et al., 2005; Neto et al., 2010; Junior et al., 2013). The synchronic approach, on
213	the other hand, is simpler, lower-cost, and less time-consuming, but comes with more uncertainty they
214	may overlook the effects of NT systems over time since it is impossible to eliminate all environmental
215	factors other than the impacts of NT systems that influence SOC and N content because of the high spatial

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216 <u>variability of land use history prior to the conduct of the experiments (Neto et al., 2010; Junior et al.,</u>
217 <u>2013</u>). A change in soil bulk density is often observed when comparing CA and NT systems to CT, due
218 to differences in tillage but also to root systems of cover crops. It is therefore required to estimate SOC
219 change using an equivalent soil mass approach instead of a fixed depth approach <u>(Ellert and Bettany,</u>
220 1995).

221 Cambodian soils are seriously threatened by inappropriate agricultural systems. The returns on taking 222 actions against land degradation are estimated at 3 US dollars for every dollar invested in restoring 223 degraded land in Cambodia, Therefore, taking actions to reverse the trend of soil degradation through 224 restoration and adopting sustainable agricultural management practices highlights the strong economic 225 benefits while combating soil degradation in the country (UNCCD, 2018). Since 2004, the conservation 226 agriculture research for development programs have been initiated by the joint-collaboration between the 227 General Directorate of Agriculture (GDA) and Centre de Coopération Internationale en Recherche 228 Agronomique pour le Développement (CIRAD), France. CA and NT cropping systems have been 229 promoted to smallholders in various agroecosystems in the countryCambodia since 2009. The early 230 effects of NT cropping systems on soil health, and SOC sequestration storage have been reported in 231 several studies (Hok et al., 2015, 2018, 2021; Pheap et al., 2019; Suong et al., 2019; Sar, 2021; Koun et 232 al., 2023), however, the information on the impact of long-term NT systems on the changes in SOC and 233 TN stocks remainss scarce in the country as well as in Southeast Asia. There is a need to document the 234 long-term changes in SOC and TN stocks under CA and NT cropping systems to fill in the knowledge 235 gaps as well as provide robust evidences to land use planners and policymakers. This could be profitable 236 not only for Cambodia but also for other countries in the whole region.

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237	Therefore, this study aimed to quantify the impacts of CT and different NT cropping systems on the
238	changes in SOC and total nitrogen (TN) stocks and fractions over time (2011-2021) in Cambodia's
239	tropical red Oxisol using diachronic and equivalent soil mass (ESM) approaches. We hypothesized that
240	implementation of the three core technical principles of CA would significantly enhance the SOC stocks,
241	both in the POM and MAOM size fractions, including in the subsoils over time. In addition, calculating
242	SOC stock using the diachronic approach would prevent a biassed estimation of the SOC accumulation
243	when compared to the synchronic approach.
244	2 Materials and Methods
245	2.1 Study site description
246	The study was conducted at Bos Khnor Conservation Agriculture Research Station, the oldest CA
247	research station in Southeast Asia, which belongs to the General Directorate of Agriculture (GDA),

248 Department of Agricultural Land Resources Management (DALRM). It is located in Chamkar Leu

249 district, Kampong Cham province (12°12'31.0"N 105°19'07.0"E, 118 m above sea level). The details of

250 the study site were reported in Hok et al., (2015). Briefly, the site was the natural tropical rainforest, which

251 was then converted to perennial cropland in 1937. The crops included cashew, coffee, mango, mulberry,

252 avocado, and rubber, which were planted soon after forest clearance. Because of the civil war (Khmer

Rouge) between 1970 and 1982, the area was abandoned and taken over by several tree species, such as

254 Tetrameles nudiflora R Br., Nauclea officinalis L., Cassia siamea (Lam.) H.S.Irwin & Barneby Lam., and

255 Leucaena leucocephala (Lam.) de Wit(L.) Benth., which grew naturally. The farming was resumed, and

cotton (Gossypium hirsutum L.) and banana (Musa acuminata spp.) were planted from 1982 to 2000.

257 From 2000 to 2009, successive annual crops per year of cotton, followed by mung bean (Vigna radiata

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258	(L.) R. Wilczek), and sesame (Sesamum indicum L.), followed by soybean (Glycine max L.), were rotated
259	under conventional plough-based management before the establishment of the three experiments. Mineral
260	fertilizers such as NPK (15-15-15), ammonium phosphate (16-20-0), and potassium chloride (0-0-60)
261	were applied to the crops without lime application. Tthe soil of the study site is classified as a red Oxisol
262	(USDA, 1999) or a Ferralsol in the world reference base (WRB) for soil resources (IUSS Working Group
263	WRB, 2015), with 1.3% sand, 29% silt, and 69% clay in the $0-20$ cm and gradually increasing with soil
264	depth to 78 % clay at 20-100 cm. The clay fraction is mainly made of kaolinite (Hok et al., 2015). The
265	land on the site is flat, the land slope $is < 1\%$. Prior to the establishment of the three experiments in
266	2009, the average SOC and TN stocks in the 0–20 cm layer were 33.6 Mg C ha ⁻¹ and 3.33 Mg N ha ⁻¹ ,
267	respectively. The research site's climate is defined as tropical monsoon, corresponding to the Am Köppen
268	climate classification, with two main seasons: the wet season from May to October and the dry season
269	from November to April. The mean annual temperature from 2009–2021 was 27.5°C, while the average
270	annual minimum and maximum temperatures were 22°C and 35°C, respectively. The annual rainfall
271	during-from 2009–202the last-1 3 years ranged between 1,650 and 2,000 mm.
272	2.2 Experimental design, treatment description, and crop management
273	The detailed history of the research site, experimental design, treatment description, and fertilizer
274	application were reported in Hok et al., (2015) and Pheap et al., (2019). Our study covers three separate

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
MaiEx, SoyEx, and CasEx, respectively. These represent the most important annual upland crops in

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279	Cambodia as well as in some Southeast Asian countries. Each The-experiments is are arranged in a
280	randomized complete block design (RCBD) with three replicates. The elementary plot dimensions are 8
281	m x 37.5_m, equivalent to 300 m ² . Each experiment consists of threefour (4)-treatments including:; (1i)
282	monocropping under conventional tillage (CTM), in which the main crops, i.e., maize (Mz), soybean (Sb),
283	and-or_cassava (Cs), wereare mono-cropped with land preparation done by disc ploughwing (CTM-Mz,
284	CTM-Sb, and CTM-Cs):: (2ii) monocropping under no-tillNTage mulch-based cropping systems with
285	the use of cover crops (NTM), in which the main crops (maize, soybean, and or cassava) wereare cropped
286	in a one-year frequency pattern under CA management (NT1 Mz, NT1 Sb, and NT1 Cs) with no soil
287	tillage along and with addition of cover crops (NTM-Mz, NTM-Sb, and NTM-Cs)-; ands; (3iii) and (iv):
288	NT were the bi-annual rotation systems of in which the main crops under NT systems with the use of
289	cover crops (NTR), where the main crops were presented every year in two separate elementary plots
290	designated as NTR1 and NTR2(i.e., soybean and cassava), were grown in a bi-annual rotation with maize
291	in the case of SoyEx (NT2 Sb, NT3 Sb) and CasEx (NT2 Cs, NT3 Cs), respectively, while the maize
292	was grown in a bi-annual rotation with soybean in the case of MaiEx (NT2-Mz, NT3-Mz) under CA
293	management along and with addition of cover crops. For treatment (3) of SoyEx and CasEx, represented
294	by NTR1-Sb and NTR1-Cs, respectively, the main crops (i.e., soybean and cassava) were grown in a bi-
295	annual rotation with maize, represented by NTR2-Sb and NTR2-Cs for SoyEx and CasEx, respectively.
296	For the treatment (3) of Mai-Ex, the main crop (i.e., maize represented by NTR1-Mz) was grown in a bi-
297	annual crop rotation with soybean, s represented by NTR2-Mz (Table 1). Under all the NT systems, the
298	species, sowing dates, and methods of cover crop establishment varied depending on the design of
299	treatments for each experiment, the types and cycles of the main crops, and the species and cycles of the

300	cover crops (Table 1). For instance, stylo (Stylosanthes guianensis (Aublet) Sw.) and Brachiaria
301	(Brachiaria ruziziensis R.Germ. & C.M.Evrard) were associated with rice and soybean, respectively, by
302	manual broadcasting at the full flowering stage of rice before the end of September and at the first yellow
303	leaves of soybean in the mid of October. Stylo was associated by line sowing with a NT planter at the
304	same date of maize cultivation and 20 days after planting for cassava. In addition, if the development
305	and/or density of the cover crop sown the previous year were considered insufficient, short cycle cover
306	crop species, i.e., pearl millet (Pennisetum typhoides (L.) Morrone) or sorghum (Sorghum bicolor (L.)
307	Moench), was sown alone or mixed with cowpea (Vigna unguiculata (L.) Walp. and sumnnhemp
308	(Crotalaria juncea L.) at the beginning of the rainy season (in the first week of May). Cover crops were
309	then grown for 60-75 days to increase the biomass inputs prior to the cultivation of the main cycle of rice.
310	soybean, or maize (Table 1).
311	The establishment and harvest of the main crops varied depending on the species. For maize, upland rice,
312	and soybean, with a life cycle of approximately 110-120 days, these crops were mainly seeded between
313	the last week of June to mid-July and harvested between mid-October and mid-November, whereas
314	cassava was planted in early May and harvested around 10 months old in the mid-February of the
315	following year.
316	For main crop residue management in MaiEx and SoyEx, all crop residues were retained in the soil in all
317	the tillage systems. In CasEx, under CTM-Cs, all the cassava fallen leaves and branches were retained in
 318	the soil, while 100% of the caseava main stems and original cuttings were completely removed from the
510	the son, while 100% of the cassava main stems and orginal cuttings were completely removed nom the

the cassava fallen leaves and branches were returned to the soil, while 50% of the cassava main stems

319

plot after harvest-under CT-Cs, representing standard farmers' practices. For all the NT-Cs systems, all

321 and 100% of the original cuttings were retained in the soil and then crimped to speed up the decomposition 322 process and facilitate field operation implementations in the following cropping season. The residues of 323 all the cover crops were left as mulch under all the NT systems in all the experiments. 324 There were a few adjustments on the cropping systems over the experimental period from 2009 to 2021, 325 especially with the use of cover crops, crop varieties, and mineral fertilizer application types and rates. 326 Details of main crops and cover crop successions and the cumulative amount of the aboveground biomass-327 C and N inputs from the crops residues are presented in Table 1. The C inputs were estimated from the 328 dry aboveground biomass inputs recorded prior to the termination of the cover crops and at grains/tuber 329 harvest for the main crops. The C and N inputs from the root systems were not recorded nor even estimated 330 based on literature. In the case of missing data of aboveground biomass, the amount of biomass was 331 estimated using the average of recorded data over time as reference in the case of cover crops and grain 332 and/or tuber-aboveground biomass ratio for the main crops (e.g. rice, maize, soybean and cassava). In 333 addition, the cumulative and annual N inputs were estimated from the amount of the cumulative and 334 annual C inputs, respectively, by applying available C/N ratio values of each plant species that were 335 vielded-obtained from the C and N concentration analysis by dry combustion.

For land preparation, the CT<u>M</u> plot<u>s wereis</u> 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), <u>s</u>Sesame (*Sesamum indicum* L.)-and mung bean (*Vigna radiata* (L.) R. Wilczek)-were sown manually under CT<u>M</u> 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<u>M</u> plots remained fallow with

342	the growth of natural grasses and broad leaves until the main cycle crops. These cropping systems
343	represent the standard farmers' practices. Under the NT systems (NTM4, NTR12 and NTR23), a long
344	cycle cover crop i.e., stylo (Stylosanthes guianensis (Aubl.) Sw.) was used as a cover crop and grown in
345	association with the main crops. This cover crop was sown in the middle of the inter-row at 0, 15, 35 days
346	after the sowing of the main crops, i.e., maize, cassava, rice, respectively, and by seed broadcasting at the
347	first yellow leaves of soybean, approximately 4 weeks before harvest. In addition, if the development
348	and/or density of the cover crop sown the previous year was considered insufficient, pearl millet
349	(Pennisetum typhoides (L.) Morrone) or sorghum (Sorghum bicolor (L.) Moench) was sown alone for the
850	treatments planted with soybean or mixed with sunnhemp (Crotalaria juncea L.) and cowpea-(Vigna
351	unguiculata (L.) Walp.) for the treatments planted with maize at the beginning of the rainy season as
352	short-cycle cover crops. The cover crops were then grown for 60-75 days prior to the main cycle of rice,
853	maize, or soybean. The main crops (rice, maize and soybean), both under $CT\underline{M}$ and <u>all the NT</u>
354	management systems, as well as the cover crops (at the beginning of the rainy season) were sown by a
355	NT planter (Fitarelli pulled by power tiller, Vence Tudo, or Seamato lifted or pulled by tractor). From
356	2009 to 2020, cassava was planted along the furrows drawn by chiselling at 0.8 m spacing to
357	approximately 20 cm depth, and then it was planted by a NT cassava planter (Planticenter) in 2021. Under
358	the NT systems, the cover crops were terminated by crimping followed by the application of a mix of
359	non-selective herbicides, i.e., glyphosate [N (phosphonomethyl) glycine] and 2,4-D [2,4-dichloro-
360	phenoxyacetic acid], at a rate of 960 and 720 g active ingredient (a.i) ha ⁻¹ , respectively.
L	

Since 2009, soil amendment was done with thermo-phosphate (16% P₂O₅, 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

363	applied with different rates of N, P, K depending on the types and phenological stage of each main crops		
364	using diammonium phosphate (18% N, 46% P2O5), ammonium phosphate sulphate (16% N, 20% P2O5),		
365	potassium chloride (60% K_{2O}), and urea (46% N). The application of the fertilizer inputs to each main		
366	crop are detailed in Table S12 in the supplementary materials.	Formatted: Font color: Auto	٦

Experiments	Crop sequences from 2009 to 2021 ^b	C input (M	lg ha ⁻¹)	N input (Mg ha ⁻¹)		
cropping systems ^a		Cumulative	Annual	Cumulative	Annual	
/laiEx						
<u>CTM-Mz</u> CT- Az	$\frac{\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{M}\mathbf{z}-}{\mathbf{M}\mathbf{z}\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{M}\mathbf{u}/\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{R}-\mathbf{M}\mathbf{z}-}$	28.60	2.20	0.64	0.05	
<u>ITM-</u> //z NT1_Mz	$\frac{Mz}{Mi/\mathbf{R} - Mi/\mathbf{R} - Mi/\mathbf{R} - St/\mathbf{R} $	67.70	5.21	1.50	0.12	
	Mi/R St/R St/R <th< td=""><td></td><td></td><td></td><td></td></th<>					
<u>NTR1-</u> <u>Az</u> NT2-Mz	$\frac{Mi/\mathbf{R} - Mi + Su + St/\mathbf{Mz} - Mi + Su + St/\mathbf{R} - St/\mathbf{Mz} + St - St/\mathbf{R} + St - St/\mathbf{Mz} + St - St/\mathbf{Mz}$	73.08	5.62	1.62	0.12	
	<u>Mi+Su+Co/Mz - So+Su+Co/Sb</u> Mt/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					
<u>VTR2-</u> <u>1z</u> NT3-Mz	$ \begin{array}{l} \underline{Mi/\mathbf{Mz} - Mi + P + St/\mathbf{R} - Mi + Su + St/\mathbf{Mz} + St - St/\mathbf{R} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + St - St/\mathbf{Mz} + St - St/\mathbf{Mz} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + St - St/\mathbf{Mz} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + St - St/\mathbf{Mz} + St - \\ \underline{St/\mathbf{R} + \\ \underline{St/\mathbf{R} + St - \\ \underline{St/\mathbf{R} + \\ St/\mathbf$	70.12	5.39	1.56	0.12	
ovEx	So+Su/R St/Mz+St So+Su+Co/Sb Mi+Su+Co/Mz					
	<u>Sb – Sb – Se/Sb – Sb – Se/Sb – Sb –</u>	23.18	1.78	0.52	0.04	
<u>TM-</u> hNT1_Sb	$\frac{\mathbf{Sb}}{Mi/\mathbf{Sb}+Br - Mi/\mathbf{Sb}+Br - Mi/\mathbf{Sb}+Br - Mi/\mathbf{Sb}+St - St/\mathbf{Sb}+St - St/$	65.09	5.01	1.45	0.11	
2.111-00	<u>Sol-Sol-Sol-Sol-Sol-Sol-Sol-Sol-Sol-Sol-</u>					
<u>FR1-</u>	$\begin{array}{llllllllllllllllllllllllllllllllllll$	71.13	5.47	1.58	0.12	
<u>1412-00</u>	$\frac{30+50t}{10} \frac{Mz}{S} = \frac{30+50t}{50} \frac{SD}{S} = \frac{30+50t}{50} \frac{Mz}{S} = \frac{SD+50t}{50} \frac{Mz}{S} = \frac{SD+50t}{50} \frac{SD}{S} = \frac{SD+50t}{50} \frac{SD+5t}{S} = \frac{SD+50t}{50} \frac{SD+5t}{S} = \frac{SD+50t}{50} \frac{SD+5t}{S} = \frac{SD+50t}{50} \frac{SD+50t}{S} = \frac{SD+50t}{50} \frac$					

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NTR2-	Mi/Mz+Br - Mi/Sb+St - Mi+Su/Mz+St - St/Sb+St - So+Su/Mz -	78.94	6.07	1.75	0.13
<u>SbNT3-Sb</u>	So+Su/Sb - So+Su/Mz - So+Su/Sb - So+Su/Mz - So+Su/Sb -				
	<u>So+Su/Mz – So+Su+Co/Sb – Mi+Su+Co/MzMi/Mz+Br – M/Sb+St</u>				
	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				
CasEx					•
CTM-CsCT-	Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-Cs-C	17.64	1.36	0.39	0.03
Cs	Cs Cs Cs				
NTM-	$\underline{\mathbf{Cs}} + \underline{St} - \underline{St}/\underline{\mathbf{Cs}} + \underline{St} - \underline{St}/\underline{\mathbf{Cs}} + \underline{St} - \underline{\mathbf{Cs}} + \underline{St} - $	46.92	3.61	1.04	0.08
CsNT1-Cs	$\underline{Cs-Cs-Cs-Cs-Cs-Cs+St-St/Cs+St-St/Cs+St-St/Cs+St-Cs+St}$				
NTR1-	$\underline{\mathbf{Cs}} + St - Mi + \underline{\mathbf{Mz}} + St - St/\underline{\mathbf{Cs}} + St - Mi + Su/\underline{\mathbf{Mz}} + St - St/\underline{\mathbf{Cs}} + St - St/\underline{\mathbf{Mz}} + St - St/\mathbf{$	64.25	4.94	1.43	0.11
CsNT2-Cs	St/Cs - St/Mz + St - St/Cs - So + Su/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - So + Su/Cs - So + Su/Cs - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su/Cs - Mi + Su + Co/Mz - So + Su +				
	<u>CsCs+St Mi+Mz+St St/Cs+St Mi+Su/Mz+St St/Cs+St</u>				
	St/Mz+St St/Cs St/Mz+St St/Cs So+Su/Mz So+Su/Cs				
	Mi+Su+Co/Mz—Cs				
NTR2-	$\underline{Mi}/\mathbf{Mz} + St - St/\mathbf{Cs} + St - \underline{Mi} + Su/\mathbf{Mz} + St - St/\mathbf{Cs} + St - \underline{Mi} + Su/\mathbf{Mz} + St - \underline{Mi} + Su/\mathbf{Mu} + Su/$	67.10	5.16	1.49	0.11
CsNT3-Cs	$\underline{St/Cs} + \underline{St} - \underline{St/Mz} + \underline{St} - \underline{St/Cs} - \underline{So} + \underline{Su/Mz} - \underline{So} + \underline{Su/Cs} - \underline{So} + \underline{Su/Mz} - \underline{Cs}$				
	<u>– Mi+Su+Co/Mz</u> 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				
	<u>So+Su/Mz Cs Mi+Su+Co/Mz</u>				

^aMaiEx: maize-based cropping system trial; SoyEx: soybean-based cropping system trial; and CasEx: cassava-based cropping system trial; CT<u>M</u>: <u>monocropping under conventional tillage;</u> NT<u>M</u>: <u>monocropping under NT systems with no-till mulch-based cropping systems associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems under NT systems with no-till mulch-based cropping systems associated with different crop sequences. <u>Another Regulations associated with different crop sequences</u>.</u>

^bBr: 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.).; The letters in bold, underlined, and italicized indicate the main crops, cash crops, and cover crops, respectively. "} 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.

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Annual mineral	Crops	Year													Total fortilizor
(kg-ha ⁻¹)*		2009	2010	2011	2012	2013	201 4	2015	2016	2017	2018	2019	2020	2021	input
N in CO(NH ₂) ₂	Cassava	92	69	69	69	69	69	69	69	69	69	69	69	69	920
	Maize	<u>92</u>	69	69	69	69	4 6	4 6	4 6	4 6	4 6	4 6	4 6	4 6	736
	Rice	69	4 6	4 6	4 6	4 6	4 6	4 6			529				
	Soybean	23	23	23	23	23									445
N in (NH₄)₂SO₄	Maize						24	2 4	2 4	2 4	24	2 4	2 4	2 4	192
N in (NH4)2HPO4	Soybean						18	18	18	18	18	18	18	18	1 44
P_2O_5	All crops	80	32	32	32	32	32	32	32	32	32	32	θ	0	400
P in (NH₄)₂SO₄	Maize						30	30	30	30	30	30	30	30	240
P in (NH₄)₂HPO₄	Soybean						4 6	46	4 6	46	4 6	46	46	4 6	368
<mark>K₂</mark> ⊖	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	4 20
	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

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

 $\frac{\text{CO(NH}_2)_2: \text{Urea} (46.0.0); (\text{NH}_4)_2\text{SO}_4: \text{Ammonium sulphate} (16.20.0); (\text{NH}_4)_2\text{HPO}_4: \text{Diammonium phosphate} (18.46-0); P_2O_5: \text{Thermo phosphate} (0.18.0); \text{and } K_2O: \text{Potassium chloride} (0.0.60).}$

369 2.2472.3 Soil sampling and processing

The study was a diachronic analysis from 2011 to 2021. In 2009, prior to the establishment of the 370 371 experiments, soil and bulk density (pb) samples were collected as the pre-experiment (PE) from three 872 randomly selected sampling points per replicate of each experimental location at four depths: 0-5, 5-10, 373 10-20, and 20-30 cm. The individual soil samples from the same depth and replicate were composited, 374 resulting in three composites per depth and per experiment. The composite samples were oven dried at 375 40°C and sieved through a 2mm mesh for chemical property analysis. Bulk density samples were 376 collected using core samplers of 5 cm in diameter and 5 cm in height, oven dried at 105°C for 48 h. The 877 SOC and TN stocks of PE in 2009 in the top 0–20 cm were 33.3, 35.0, and 32.4 Mg C ha⁻¹ and 3.34, 3.41, 378 and 3.26 Mg N ha⁻¹ in MaiEx, SoyEx, and CasEx, respectively (Fig. S1). 379 In November 2011, soil sampling was conducted to assess the early effects of tillage systems on soil 380 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 ob sample collection. Individual 381 382 samples for chemical analysis were collected from two undisturbed sides of each pit at 0-5, 5-10, 10-20, 383 20-40, 40-60, 60-80, and 80-100 cm. The individual samples from the same depth and the same pit were 384 composited, and as a result, two composite samples per layer were collected per elementary plot. The 385 composites were oven-dried at 40°C before being softly disrupted, sieved through a 2-mm sieve, and 386 homogenized. Soil bulk density samples were taken from the same two undisturbed sides of each pit at 387 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 388

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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 pb, SOC and N concentrations and 395 396 stocks ten years after the study conducted by Hok et al., (2015). The samples were collected at the same 397 7 layers. From each treatment and replicate, we collected four individual samples by an automatic soil 898 column cylinder auger (a gasoline-powered percussion hammer Cobra TT with inner diameter of 85 mm, Eijkelkamp, the Netherlands) in a diagonal "X" shape from four points within each plot, avoiding 399 400 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 401 sample collection; three individual soil samples and three pb cores were collected from three undisturbed 402 sides of the pit at each depth. Soil samples were air-dried at room temperature, gently broken down, and 403 sieved through a 2 mm mesh sieve. Finally, the seven individual samples from the same layer were mixed 404 and homogenized to make a composite sample per elementary plot. The samples of pb were oven-dried 405 at 105°C for 48 h.

406 2.2482.4 Soil organic Cand total N analyses

The <u>concentrations of</u> SOC and <u>total-T</u>N <u>concentration</u> 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.

413 2.2492.5 Soil organic C and total N stocks calculation

414 In this Oxisol, there was no coarse fraction (i.e. gravels) left after sieving at 2 mm. Therefore, the bulk 415 density of soil equals the bulk density of fine earth. To avoid inaccurate stock calculation due to 416 differences in bulk density between treatments when using the fixed depth method, the equivalent soil 417 mass (ESM) approach was applied to compute SOC and TN stocks (Ellert and Bettany, 1995; Von Haden 418 et al., 2020; Fowler et al., 2023). Since the pb of the treatments differed between the two sampling years 419 (2011 and 2021) and the reference vegetation at each sampling depth (Table S_{21}^{-1} in the supplementary 420 materials), we defined the reference soil mass as the lowest soil mass observed at each sampling depth, 421 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, 422 40-60, 60-80, and 80-100 cm, respectively). We applied these reference soil masses to compute the SOC 423 424 and TN stocks in 2021 and recalculated the stocks of the PE, RV and the treatments of the three 425 experiments collected in 2009 and 2011.

- To correct for differences in ρb , SOC and <u>TN</u> stocks were computed according to Eq. 1 and 2.
- 427 $M_{(Soilmin,i)} = \rho b_{(i)} \times T_{(i)} \times 1000$

(eq.1)

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428	$SOC \text{ or } TN \text{ stock} = \sum (i=1)^{n} n \left[(M_{(soilmin,i)} \times \text{ conc.}_{(i)}) + ((M_{(soil,i)} - M_{(soilmin,i)}) \times \text{ conc.}_{(i-1)}) \right] \times Conc{(i-1)} $								
429	0.001(eq. 2)								
430	Where: $M_{(soilmin,i)}$ is the minimal soil mass per unit area in the ith layer (Mg ha ⁻¹) recorded over the								
431	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								
432	of the ith layer (m). $conc{(i)}$ is the concentration of SOC in ith layer. $conc{(i-1)}$ is the concentration of								
433	SOC in i - 1th layer. $M_{(soil,i)}$ is the designated soil mass of each layer (i.e., the maximum soil mass). The								
434	numbers 1000 and 0.001 are unit conversion coefficients.								
435									
436	We defined deltastock (Δ) of SOC and TN, as the stock change within the same treatment and depth								
437	between 2021 and 2011 sampling years (diachronic) and calculated it as follows:								
438	$\Delta SOC \text{ or } TN \text{ stock. } diach = SOC \text{ or } TN \text{ stock}_{treat.ment} (i)_{2021} -$								
439	SOC or TNstock _{treat.ment} (i)2011 (eq.3)								
440	Where: i represents the treatments.								
441									
442	To compare the synchronic and diachronic approaches for SOC stock change, the stock change estimated								
443	by the synchronic approach was computed as follows using the $CT\underline{M}$ treatment as the control treatment:								
444	$\Delta SOC stock. synch$								
445	$= SOCstock_{NT(i)2021} - SOCstock_{CTM2021} - (eq.4)$								
446	Where: NT(i) represents NTM4, NTR12, and NTR23 treatments.								

The SOC and <u>TN</u> stock change (accumulation or loss) rates (Mg C or N ha⁻¹ yr⁻¹) of each treatment were calculated by dividing Δ SOC or <u>TN</u> stock by the number of years between the 1st and 2nd samplings (10 vears):

- 450 SOC or TN stock chang-e rate_{treatment(i)} =
- $451 \quad \frac{\Delta SOC \text{ or } TN \text{ stock}_{treatment(i)}}{10} \quad -- \quad (eq. 5)$

452 2.2502.6 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 453 454 treatments and seven depths. The particle-size fractionation was implemented in accordance with the 455 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. 456 457 The sample was then horizontally shaken at 100 rpm for 8 hours with three 10-mm-diameter agate balls. 458 The soil mixture was wet-sieved with deionized water through a 53-µm sieve to get the proportion of 459 particulate organic matter (POM) sized between 53µm and 2,000µm. The <-53-µm fraction was 460 flocculated with 2-g CaCl₂ in a 1-L glass cylinder and left overnight for sedimentation. The supernatant 461 was syphoned after full sedimentation. This <-53-µm fraction is made up of mineral-associated organic 462 matter (MAOM). The two fractions were oven-dried at 40°C until reaching constant weight and finely 463 ground for determining SOC and TN concentrations by dry combustion using the LECO® CHN628

464 analyzer at the Sustainable Agroecosystems Lab, ETH Zurich University, Switzerland.

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465 2.2512.7 Statistical analysis

466 Statistical analysis was condmputed using R software, version 4.3.1 (Core Team, 2023). Linear mixed 467 models (ImerTest package) were fitted on all data: sampling years, soil depths, and treatments were 468 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 469 470 homoscedasticity of the data. A diachronic approach was used to assess statistical significance between 471 the two sampling years (i.e., 2021 vs. 2011) of the same treatment at the same soil depth by analysis of 472 variance with Fisher tests (degrees of freedom calculated by Satterthwaite method), computing of 473 estimated marginal means (EMMs) and p-value adjustment using the Tukey method. The same approach 474 was used to compare SOC stocks between treatments in the same sampling year, calculated at equivalent 475 soil mass and using the synchronic approach. In addition, we applied the same statistical procedures to 476 assess the statistical significance of cumulative SOC and TN stocks.

477	3 Results	 Formatted: Font color: Auto
478	The effects of cropping systems on the concentrations, stocks of SOC and <u>TN</u> as well as their fractions in	
479	the physical size classes between 2011 and 2021 varied among the three experiments and across the soil	
480	profile.	
1		
481	<u>3.1 Impacts of tillage and c</u> Cropping system <u>effects</u> on SOC and N concentrations and stocks	
482	3.13.1.1 SOC concentration	Formatted: Heading 3, No bullets or numbering
483	The <u>SOC</u> concentrations of <u>SOC</u> all the treatmentsd N in 2011 and 2021 wasre presented highest in Fig.	
484	1 and Fig. 2, respectively, with_the duplicates propysoil (0-5 cm) ded in Table S2 and decreased with 28	Formatted: Font color: Auto

486	stock change rate between 2011 and 2021, respectively. The SOC and N stocks in 2011 all the experiments	
487	(Fig.1 with re-provided in Table S 34 in the supplementary as duplication) materials.	Formatted: Font color: Auto
488		
489	The SOC concentration of all the treatments in 2011 and 2021 was highest in the topsoil (0-5 cm) and	
490	decreased with soil depth (Fig. 1A and 1B). From 2011 to 2021, SOC concentration under NT-Mz crop	
491	rotation systems (average of NT2-Mz and NT3-Mz) significantly increased by 50%, 24%, and 15% at 0-	
492	5, 5–10, and 10–20 cm depth, respectively. Significant increase was still observed under NT2 Mz at 20-	
493	40 cm depth. NT1 Mz significantly increased SOC concentration over the whole soil profile by 68%,	
494	21%, 16%, 17%, 23%, and 16% at 0 5, 5 10, 20 40, 40 60, 60 80, and 80 100 cm, respectively (Fig.	
495	1B) when compared between 2021 and 2011. In the case of CT-Mz, except for the significant increase	
496	observed in the 10-20 cm layer, the SOC concentration remained neutral at 0-10 and 20-80 cm, then	
497	significantly decreased at 80–100 cm (Fig. 1A and 1B).	
498	Over <u>a</u> 10-year <u>period</u> of historical cropping sequences from 2011 to 2021, the all the NT cropping systems	
499	had significant reffsponsects ($p < 0.05$) of SOC stock and in the increase of the distribution varied	Formatted: Font: Italic, Complex Script Font: Italic
500	depending on the tillage and cropping systems (Table 3). SOC stock under NT Mz crop rotation systems	
501	increntrationased 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 -	
502	10, and 10–20 cm depth, respectively. Unlike SOC concentration, NT1-Mz only significantly increased	
503	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,	
504	respectively. Despite CT Mz significantly accumulated SOC stock in the tilled layers (5-10 and 10-20	
I	29	

485 soil depth S3 in the supplementary materials. Table 3 and 4 show the SOC and N stocks in 2021 and the

505	cm), significant reductions were found in the 60-80 and 80-100 cm depths (Table 3). On the other hand,
506	the
507	At the first sampling in 2011, SOC stock in 0–20 cm depth under RV was significantly ($P < 0.05$) higher
508	at 17%, 21%, and 22% than under concentration under CT-Mz remained stable with exception of a few
509	significant, NT1-Mz, and NT-Mz crop rotation systems, increaspes detected in the tilled layers in ctively.
510	In 2021, SOC stock under CT-MaiEx and CasEx (Fig. 1). z and all the NT Mz did not differ (P < 0.05)
511	from RV. SOC stock was fully recovered under NT-Mz crop rotation systems and even surpassed the
512	RV's stock by +0.64 Mg C ha ⁻¹ under NT1-Mz, while the difference with RV decreased from 17 to 8%
513	under CT-Mz (Fig. 3A).
514	Considering a 100 cm as a single stratum, all the NT Mz cropping systems significantly increased SOC
515	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-
516	Mz (Table 3).
517	Over 10 years of cultivation, all the NT Mz systems significantly increased N concentration in the top 0-
518	5 cm depth (32% in NT Mz crop rotation systems and 44% in NT1 Mz), but soil N significantly decreased
519	by -24% in NTI-Mz at 40-60 cm and at least from -18 to -21% in NT-Mz crop rotation systems from 40
520	to 100 cm. In CT-Mz, soil N concentration remained neutral from 0 to 40 cm, then significantly decreased
521	in the 40–60 and 80–100 cm depths (Fig. 2A and 2B).
522	N stock in the soils under all the NT Mz systems significantly increased in the uppermost soil surface (0-
523	5 cm), remained neutral in the 5-40 cm, then significantly decreased below 40 cm (Table 4). Averaged
524	N stock in NT Mz crop rotation systems increased by 32%, equivalent to 0.3 Mg N ha ^{$+$} in the 0–5 cm,
525	whereas there was significant reduction of 16% and 23% from 40 to 100 cm. In NT1 Mz, N stock 30

30

526	significantly increased in the 0–5 cm layer by 43%, equivalent to 0.4 Mg N ha ⁻¹ , then significantly reduced
527	by 23% in the 40-60 cm. In the case of CT Mz, N stock remained constant from 0-40 cm, then
528	significantly declined from -14 to -24% from 40 to 100 cm (Table 4).
529	When RV was used as a reference (0-20 cm depth), N stock in 2011 was significantly lower by 25%,
530	22%, and 20% under CT Mz, NT1 Mz, and NT Mz crop rotation systems, respectively. In 2021, the
531	difference in N-stock decreased to 15% and 7.5% under the NT1 Mz and NT Mz crop rotation systems,
532	respectively, when compared with RV. In contrast to NT-Mz, N stock did not change under CT-Mz in
533	2021 (Fig. 3D).
534	Although not significant, after nearly a decade of rice based and the recent shift to maize based systems,
535	soil N stock in the whole profile (0 100 cm) showed a decreasing trend by 11%, 6%, and 5%,
536	equivalent to annual loss rates of -0.11, -0.06, and -0.06 Mg N ha ⁺ yr ⁺ under CT-Mz, NT1-Mz, and NT-
537	Mz biannual rotation systems, respectively (Table 4).
538	3.1.2 Soybean based experiment
539	After 10 years of implementation, significant increase ($p < 0.05$) in SOC concentration was only observed
540	under all the NT-Sb cropping systems (NT1-Sb, NT2-Sb, and NT3-Sb) in the top 0-5 cm with a similar
541	increase amount of ~7.5 g C kg ⁺ soil. SOC concentration in CT-Cs remained stable across the whole
542	profile (Fig. 1C and 1D).
543	SOC stock significantly increased in the surface layers (0-10 cm) under all NT Sb systems (Table 3).
544	SOC stock significantly increased by 3.6 Mg C ha 1 in the 0-5 cm in NT1-Sb. NT-Sb crop rotation

545 systems (average of NT2-Sb and NT3-Sb) significantly accumulated SOC stock by 3.55 and 1.75 Mg C

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546	ha ⁺ in 0 5 and 5 10 cm, respectively, along with a positive trend from 10 to 60 cm depth. Unlike all the
547	NT Sb systems, the SOC stock in the CT Sb soil remained neutral across the whole profile (Table 3).
548	In 2011, RV as a reference (0-20 cm) significantly stored 13%, 14%, and 16% higher SOC stock than
549	CT-Sb, NT1-Sb, and NT-Sb bi-annual rotation, respectively. In 2021, SOC stock increased and a 100%
550	recovery was observed under NT Sb biannual rotation systems, while the differences with RV dropped
551	to 9% and 5% under CT Sb and NT1-Sb, respectively, although not significant (Fig. 3B).
552	Considering the whole profile (0-100 cm), after 10 years of cultivation, the study showed that all the NT-
553	Sb systems increased SOC stock with annual accumulation rates ranging from 0.65 to 1.00 Mg C ha ⁺ yr
554	⁴ -although non-significant difference was detected (Table 3).
555	From 2011 to 2021, all the NT-Sb systems significantly increased soil N concentration in the top 0-5 cm
556	with a similar increase of ~0.46 g N kg ⁺ soil (Fig. 2C and 2D). Noticeably, significant decreases in N
557	concentration were observed in the 40 60, 60 80, and 80 100 cm under NT-Sb bi-annual rotation
558	systems and in the 60-80 and 80-100 cm depths under NT1 Sb (Fig. 2C and 2D). In contrast to NT Sb
559	systems, N concentration remained constant across the whole profile in soybean monocropping under
560	conventional plough-based tillage (CT-Sb) (Fig 2D).
561	Over 10 years of cultivation, the N-stock significantly increased in a similar amount of 0.2 Mg N ha ⁺
562	under all the NT Sb systems in the top 0-5 cm. The N stock under all the NT Sb systems remained stable
563	from 5 to 60 cm, then significantly decreased in the 60-80 and 80-100 cm depths (Table 4).
564	In 20211, RV's soil (0-20 cm) significantly stored 23%, 20%, and 18% higher N stock than CT-Sb, NT1-
565	Sb. and NT-Sb crop rotation systems, respectively. In 2021, N stock increased and the differences with

566	RV reduced to 16%, 18%, and 11% under CT Sb, NT1 Sb, and NT Sb crop rotation systems, respectively,
567	although not significant (Fig. 3E).
568	Measured in the whole profile (0 100 cm), soybean monocropping under NT systems (NT1-Sb)
569	significantly decreased N stock with an annual loss rate of -0.1 Mg N ha ⁺ yr ⁺ , while NT-Sb bi-annual
570	rotation systems exhibited a decrease trend of N stock with an annual loss rate of $0.06 \text{ Mg N ha}^4 \text{ yr}^4$
571	(Table 4). Despite the significant increase in the 10-20 and decrease in the 60-80 cm, N stock in CT Sb
572	remained stable when considering the whole profile 0-100 cm (Table 4). the monocropping of main crops
573	under NT systems in MaiEx (NTM-Mz) and CasEx (NTM-Cs) exhibited a similar trend in increasing
574	SOC concentration significantly ($p < 0.05$) across the soil profile compared to 2011 (Figs. 1B and 1F).
575	The SOC concentration under NTM-Mz increased by 68%, 21%, 16%, 17%, 23%, and 16% at 0-5, 5-
576	10, 20-40, 40-60, 60-80, and 80-100 cm, respectively (Fig. 1B). The significant increase in SOC
577	concentration under NTM-Cs was detected from 0 to 80 cm with a gain of 26%, 20%, 19%, 22%, 18%,
578	and 10% in the 0-5, 5-10, 10-20, 20-40, 40-60, and 60-80 cm, respectively (Fig. 1F).
579	When compared to 2011, the bi-annual rotation of main crops under NT systems in MaiEx (NTR1-Mz
580	and NTR2-Mz) and CasEx (NTR1-Cs and NTR2-Cs) significantly ($p < 0.05$) increased SOC
581	concentration from the surface down to subsoil depth in 2021 (Figs. 1B and 1F). On average NTR-Mz
582	(average of NTR1-Mz and NTR2-Mz), significantly increased SOC concentration by 50%, 24%, and 15%
583	at 0-5, 5-10, and 10-20 cm depth, respectively. Significant increase was still observed under NTR1-Mz
584	at 20-40 cm depth (Fig. 1B). In 2021, among the two treatments of NTR-Cs crop rotation systems (NTR1-
585	Cs and NTR2-Cs), NTR2-Cs significantly increased SOC concentration from the top 0 to 60 cm by 30%,
586	12%, 13%, 23%, and 15% in the 0–5, 5–10, 10–20, 20–40, and 40–60 cm, respectively, while a significant 33

587	decrease of	-13%	was r	recorded	in the	80-	100	cm	depth	(Figs.	1E and	1F)	. Under	NTR	1-Cs.	, sig	nifica	ant

- increases in SOC concentration were observed up to 40 cm depth with a gain of 24%, 10%, and 23% in
- 589 0-5, 5-10, and 20-40 cm, respectively, with a significant decrease by -12% in 80-100 cm depth (Figs.
- 590 <u>1E and 1F).</u>
- 591 Unlike MaiEx and CasEx, the significant increase (p < 0.05) in SOC concentration under all the NT
- 592 cropping systems in SoyEx (NTM-Sb, NTR1-Sb, and NTR2-Sb) in 2021 was only observed in the top 0-
- 593 <u>5 cm with a similar increase amount of ~7.5 g C kg⁻¹ soil (Fig 1D).</u>
- 594 Over a decade of monocropping of main crops under conventional tillage in all the experiments (CTM-
- 595 Mz, CTM-Sb, and CTM-Cs), the SOC concentration remained neutralstable overall, with the exception
- of a few significant increases detected in the tilled layers in MaiEx (CTM-Mz at 10–20 cm) and CasEx
- 597 (CTM-Cs at 0–5 and 5–10 cm) (Figs. 1B, 1D, and 1F).



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Figure 1. SOC concentration <u>distribution across the soil profile</u> (0_-100 cm) <u>of the treatments in 2011</u> and <u>2021</u>-under different <u>exeroperiping systements in 2011</u> and 2021. <u>CTM</u>: monocropping under conventional tillage; NTM: monocropping under NT systems with no--till mulch-based cropping systems associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems

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under NT systems with no-till mulch-based cropping systems associated with different crop sequences conventional tillage; NT: no till, NT1, NT2, and NT3 refer to no till associated with different cropping systems as described in Table 1. A: MaiEx 2011 – SOC concentration of the treatments in maize-based trial measured in 2011; B: MaiEx 2021 – SOC concentration of the treatments in maize-based trial measured in 2021; C: SoyEx 2011 – SOC concentration of the treatments in soybean-based trial measured in 2021; D: SoyEx 2021 – SOC concentration of the treatments in soybean-based trial measured in 2021; D: SoyEx 2021 – SOC concentration of the treatments in soybean-based trial measured in 2021; E: CasEx 2011 – SOC concentration of the treatments in cassava-based trial measured in 2011; and F: CasEx 2021 – SOC concentration of the treatments in cassava-based trial measured in 2021. Treatment(s) in bold within the brackets indicate the gain and significant (p < 0.05) difference in concentrations between 2011 and 2021 in the same treatment at the same soil depth,

598 <u>3.1.2 SOC stock</u>

599	From 2011 to 2021	, there were significant ((p < 0.05)) increases in SOC	stock-and vertical distribution,
			-		

- which varied depending on tillage, cropping systems, and the experiments (Table 2 and Table S5). In
- 501 2021, in the case of MaiEx, the SOC stock under NTR-Mz crop rotation systems (average of NTR1-Mz
- 602 and NTR2-Mz) significantly (p < 0.05) increased by 4.6, 2.6, and 2.2 Mg C ha⁻¹ at 0–5, 5–10, and 10–20
- 603 <u>cm depth, respectively. NTR1-Mz showed a significant increase in SOC stock at a deeper profile at 20–</u>
- 40 cm, with a gain of 4.5 Mg C ha⁻¹ (Table 2 and Table S5). In the case of CasEx, the soils under NTR-
- Cs crop rotation systems (average of NTR1-Cs and NTR2-Cs) in 2021 significantly (p < 0.05) increased
- 606 SOC stock by an average of 2.4, 1.1, 1.4, and 2.9 Mg C ha⁻¹ in 0-5, 5-10, 10-20, and 20-40 cm,
- 607 respectively, but significantly decreased by an average of -0.9 Mg C ha⁻¹ in 80–100 cm (Table 2 and Table
- 608 S5). For SoyEx, NTR-Sb crop rotation systems (average of NTR1-Sb and NTR2-Sb) significantly
- accumulated SOC stock by an average of 3.55 and 1.75 Mg C ha⁻¹ in 0–5 and 5–10 cm, respectively,
- 610 along with a positive trend from 10 to 80 cm depth (Table 2 and Table S5).
- 511 Unlike SOC concentration, the significant effect of increasing SOC stock of the monocropping of main
- 612 crops under NT systems varied across the three experiments (Table 2). NTM-Cs showed the significant

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- 614 40 cm, respectively (Table 2 and Table S5). NTM-Mz significantly increased SOC stock in the surface
- b15 layers (0-10 cm) by 6.1 and 2.2 Mg C ha⁻¹ in the 0-5 and 5-10 cm, respectively, while the NTM-Sb
- 516 significantly increased the stock by 3.6 Mg C ha⁻¹ in the 0–5 cm (Table 2 and Table S5).
- 517 In the case of monocropping of main crops under conventional tillage, despite there were a few significant
- 618 increases in SOC stock were detected in the till layers in CTM-Mz (MaiEx) by 0.9 and 2.3 Mg C ha⁻¹ in
- the 5-10 and 10-20 cm, respectively, the significant decline in SOC stock was observed below 60 cm
- with the decreasing by approximately -1.2 Mg C ha⁻¹ at the 60–80 and 80–100 cm depth (Table 2). For
- 621 CasEx and SoyEx, despite the significant increase in SOC stock was observed in CTM-Cs from 0 to 20
- 622 <u>cm, the accumulation rate was 2 times lower than those NT systems, while no significant changes were</u>
- 623 recorded under CTM-Sb (Table 2).
- 624 Although non-significant, when compared with PEOver the 10-year period from 2011 to 2021,
- considering a 100 cm layer as a single stratum, all the NT cropping systems significantly increased SOC
- stock, with accumulation rates ranging from 0.86 to 1.47 and 0.70 to 1.07 Mg C ha⁻¹ yr⁻¹ Mg C ha⁻¹ yr⁻¹
- 527 for CA-Mz and CA-Cs, respectively (Table 2). Although non-significant (p > 0.05)-difference detected,
- 528 the study showed that all the NT-Sb systems increased SOC stock with annual accumulation rates ranging
- f29 from 0.65 to 1.00 Mg C ha⁻¹ yr⁻¹ (Table 2). Despite there were a few significant increases in SOC stock
- were observed under CTM, the whole profile SOC stock in all the CTM (CTM-Mz, CTM-Sb, and CTM-
- 631 <u>Cs) remained neutralstable in 2021 (Table 2).</u>

<u>xperiments^a</u>	<u>Approximate</u> <u>soil depth</u>	Cropping system	<u>1S^b</u>						
	<u>(cm)</u>	<u>CTM</u>	<u>NTM</u>	<u>NTR1</u>	<u>NTR2</u>	<u>CTM</u>	<u>NTM</u>	<u>NTR1</u>	<u>NTR2</u>
		SOC stock in 202	21 (Mg C ha ⁻¹)			SOC stock cl	hange rate 20	21-11 (Mg C	ha ⁻¹ yr ⁻¹)
MaiEx	<u>0-5</u>	<u>9.83 (±0.19)</u>	<u>15.01 (±0.38)</u> A	<u>14.03 (±0.32)</u> A	<u>13.52 (±0.54)</u> A	0.04 (±0.03)	0.61 (±0.06)	0.48 (±0.02)	0.44 (±0.02)
A	<u>5-10</u>	<u>10.16 (±0.18)</u> <u>A</u>	<u>10.91 (±0.11)</u> A	<u>11.16 (±0.06)</u> A	<u>10.89 (±0.46)</u> <u>A</u>	0.09 (±0.03)	0.22 (±0.01)	0.23 (±0.00)	0.22 (±0.06)
A	<u>10-20</u>	<u>18.28 (±0.26)</u> <u>A</u>	<u>16.31 (±0.57)</u>	<u>17.08 (±0.07)</u> <u>A</u>	<u>16.32 (±0.69)</u> <u>A</u>	0.23 (±0.02)	<u>0.11 (±0.06)</u>	0.25 (±0.04)	0.19 (±0.09)
A	20-40	<u>19.90 (±0.72)</u>	<u>18.61 (±0.43)</u>	<u>20.28 (±0.70) A</u>	<u>18.64 (±0.58)</u>	<u>0.11 (±0.06)</u>	<u>0.16 (±0.07)</u>	0.45 (±0.08)	0.07 (±0.08)
A	<u>40-60</u>	<u>12.58 (±0.37)</u>	<u>12.18 (±0.25)</u>	<u>12.75 (±0.42)</u>	<u>12.31 (±0.19)</u>	<u>-0.04 (±0.03)</u>	0.08 (±0.08)	0.10 (±0.03)	0.02 (±0.05)
A	<u>60-80</u>	<u>9.01 (±0.61)</u> <u>B</u>	9.58 (±0.31)	<u>9.64 (±0.48)</u>	<u>9.33 (±0.26)</u>	<u>-0.12 (±0.02)</u>	0.07 (±0.08)	<u>-0.02 (±0.01)</u>	<u>-0.02 (±0.01)</u>
A	<u>80-100</u>	<u>7.17 (±0.66)</u> <u>B</u>	7.51 (±0.54)	7.97 (±0.38)	7.26 (±0.05)	<u>-0.13 (±0.05)</u>	0.05 (±0.08)	<u>-0.02 (±0.02)</u>	-0.06 (±0.05)
A	<u>0-100</u>	<u>86.92 (±2.18)</u>	<u>90.13 (±1.82)</u> <u>A</u>	<u>92.90 (±2.30)</u> <u>A</u>	<u>88.27 (±2.11)</u> <u>A</u>	<u>0.17 (±0.15)</u>	1.30 (±0.38)	1.47 (±0.11)	0.86 (±0.20)
SoyEx	0-5	9.45 (±.0.05)	<u>13.17 (±0.19)</u> A	<u>13.14 (±0.30)</u> A	<u>13.58 (±0.31)</u> A	0.02 (±0.03)	0.36 (±0.02)	0.32 (±0.02)	0.39 (±0.05)
	<u>5-10</u>	<u>9.95 (±0.10)</u>	9.74 (±1.45)	<u>11.05 (±0.12)</u> A	<u>10.80 (±0.21)</u> <u>A</u>	0.03 (±0.03)	0.04 (±0.12)	0.18 (±0.02)	0.17 (±0.02)
	<u>10-20</u>	18.35 (±0.12)	<u>16.79 (±1.84)</u>	<u>17.27 (±0.35)</u>	<u>17.43 (±0.43)</u>	0.11 (±0.05)	-0.02 (±0.16)	0.17 (±0.04)	0.08 (±0.07)
	<u>20-40</u>	22.34 (±0.50)	22.66 (±1.18)	<u>19.26 (±0.43)</u>	<u>19.69 (±0.75)</u>	<u>0.19 (±0.18)</u>	<u>0.17 (±0.17)</u>	<u>0.19 (±0.07)</u>	<u>0.18 (±0.13)</u>
A	<u>40-60</u>	<u>15.20 (±0.61)</u>	15.86 (±0.55)	13.00 (±0.65)	13.08 (±0.43)	0.14 (±0.18)	0.10 (±0.10)	0.13 (±0.03)	0.17 (±0.06)
	<u>60-80</u>	<u>11.16 (±0.52)</u>	11.95 (±0.62)	9.48 (±0.32)	9.32 (±0.44)	0.04 (±0.14)	0.06 (±0.04)	0.01 (±0.04)	0.04 (±0.06)
	80-100	<u>8.76 (±0.57)</u>	9.37 (±0.82)	7.03 (±0.04)	7.25 (±0.37)	<u>-0.04 (±0.11)</u>	<u>-0.05 (±0.01)</u>	-0.08 (±0.05)	<u>-0.02 (±0.02)</u>
	0-100	<u>95.22 (±2.06)</u>	<u>99.53 (±4.26)</u>	90.23 (±1.52)	<u>91.15 (±1.87)</u>	0.48 (±0.70)	0.65 (±0.58)	0.92 (±0.13)	1.00 (±0.29)
CasEx	0-5	8.44 (±0.23) A	9.48 (±0.09) A	10.68 (±0.00) A	11.67 (±0.34) A	0.09 (±0.01)	0.20 (±0.03)	0.21 (±0.01)	0.27 (±0.06)
	5-10	8.97 (±0.25) A	9.52 (±0.17) A	9.82 (±0.04) A	9.76 (±0.22) A	0.05 (±0.01)	0.16 (±0.02)	0.10 (±0.00)	0.12 (±0.03)
	10-20	<u>17.15 (±0.30)</u> A	<u>16.66 (±0.49)</u> A	<u>16.63 (±0.15)</u> A	<u>16.23 (±0.24)</u> A	0.09 (±0.03)	0.25 (±0.03)	0.09 (±0.01)	0.18 (±0.04)
	20-40	18.18 (±0.94)	<u>18.84 (±0.74)</u> A	<u>20.77 (±0.22)</u> <u>A</u>	<u>19.27 (±1.02)</u> A	-0.09 (±0.11)	0.22 (±0.06)	0.29 (±0.06)	0.29 (±0.01)
	<u>40-60</u>	<u>13.12 (±0.44)</u>	<u>13.42 (±0.36)</u>	<u>13.95 (±0.22)</u>	<u>12.99 (±0.70)</u>	<u>-0.05 (±0.11)</u>	0.14 (±0.05)	0.12 (±0.03)	0.13 (±0.02)
	<u>60-80</u>	<u>11.11 (±0.08)</u>	10.78 (±0.47)	10.61 (±0.49)	10.18 (±0.59)	0.02 (±0.08)	0.09 (±0.04)	-0.01 (±0.01)	0.04 (±0.01)
	80-100	<u>9.47 (±0.09)</u>	<u>8.71 (±0.33)</u>	<u>8.62 (±0.57)</u> B	<u>7.91 (±0.46)</u> B	0.02 (±0.04)	0.01 (±0.01)	-0.10 (±0.01)	-0.08 (±0.05)
•	0-100	<u>86.44 (±2.12)</u>	<u>87.42 (±2.52)</u> <u>A</u>	<u>91.07 (±0.92)</u> A	<u>88.01 (±3.03)</u> A	0.14 (±0.35)	1.07 (±0.18)	0.70 (±0.11)	0.95 (±0.07)
laiEx: Mai	ze-based exi	periment: Sov	Ex: Sovbean	-based exper	iment: and C	asEx: Cassa	va-based e	experiment	
TM· mon	ocropping u	nder conventio	onal tillage:	NTM: mon	ocropping up	der NT syst	tems with	no-till m	<u>.</u> ulch-hase
opping syst	eme associat	ad with differ	ant crop secu	ences and N	ITR 1 and NT	R^2 refer to b	i_annual or	on rotation	al system

Table 2. Mean SOC stocks in 2021 and SOC stock change rate between 2021 and 2011.

^a MatEx: Marze-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment. ^b CTM: monocropping under conventional tillage; NTM: monocropping under NT systems with no--till mulch-based cropping systems associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems under NT systems with no-till mulch-based cropping systems associated with different crop sequences as described in Table 1. Different uppercase letters accompanying the values indicate significant difference within the same treatment between 2011 and 2021 (diachronic) at the same soil depth at p < 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 <

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0.05 (Tukey's test). Positive values of SOC stock change rate indicate a SOC accumulation; negative values indicate a SOC loss. Values in the parentheses indicate standard errors (n=3).



3.2 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

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significant difference in concentrations between 2011 and 2021 in the same treatment at the same soil depth.

632 633 Figure 3. Changes in SOC and N stocks (Mg ha-1) at 0-20 em depth from pre-experiment (PE) in 2009, 2011, and 2021 under different eropping systems with reference vegetation (RV) 634 for comparison. CT: conventional tillage; NT: no-till, NT1, NT2, and NT3 refer to no-till

635 636 637 638	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).
639 640	3.2 CImpact of tillage and cropping systems on TN concentration and stockassava-based experiment
641	3.1.33.2.1 Total N concentration
642	Over <u>10 years of cultivation from 2011 to 2021</u> a decade, all the treatments significantly (P < 0.05)
643	increased SOC concentration in the upper layers (0-10 cm), while significant increases below 10 cm were
644	only observed under NT-Cs systems (Fig. 1F). Among the two treatments of NT-Cs crop rotation systems
645	(NT2-Cs and NT3-Cs), NT3-Cs significantly increased SOC concentration from the top 0 to 60 cm by
646	30%, 12%, 13%, 23%, and 15% in the 0 5, 5 10, 10 20, 20 40, and 40 60 cm, respectively, while a
647	significant decrease of 13% was recorded in the 80–100 cm depth. Under NT2 Cs, significant increases
648	in SOC concentration were observed up to 40 cm depth with a gain of 24%, 10%, and 23% in 0-5, 5-10,
649	and 20-40 cm, respectively, with a significant decrease by -12% in 80-100 cm depth (Fig. 1E and 1F).
650	In the case of cassava monocropping under NT systems (NTI-Cs), significant increases in SOC
651	concentration were detected from 0 to 80 cm with a gain of 26%, 20%, 19%, 22%, 18%, and 10% in the
652	0-5, 5-10, 10-20, 20-40, 40-60, and 60-80 cm, respectively (Fig. 1F). For CT Cs, SOC concentration
653	significantly increased, but at 2 times lower than that of those NT-Cs systems, by 12% and 5% in the top
654	0 5 and 5 10 cm, respectively, with a significant decrease by 10% detected in 20 40 cm depth (Fig. 1E
655	and 1F).
656	From 2011 to 2021, SOC stocks under the NT Cs crop rotation systems (NT2 Cs and NT3 Cs) increased
657	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

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658	respectively, with still a positive trend in 40 60 cm and then a significant decrease by -0.9 Mg C ha ⁻¹ in
659	80-100 cm (Table 3). Similarly, NT1-Cs significantly increased SOC stock by 2.0, 1.6, 2.5, and 2.2 Mg
660	C ha+ in 0 5, 5 10, 10 20, and 20 40 cm, respectively (Table 3). For cassava monocropping under
661	conventional tillage (CT-Cs), SOC stock significantly increased, but at 2 times lower than all the NT-Cs
662	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
663	changes were recorded below 40 cm (Table 3).
664	In 2011, with RV used as a reference (0-20 cm), SOC stock was significantly higher in RV by 23%, 29%,
665	and 22% in CT-Cs, NTI-Cs, and NT-Cs crop rotation systems (average of NT2-Cs and NT3-Cs),
666	respectively. In 2021, the difference in SOC stock with RV declined to 17%, 14%, and 10% in CT Cs,
667	NTI-Cs, and NT-Cs crop rotation systems, respectively (Fig. 3C).
668	Over a 10-year period, from 0 to 100 cm depth, SOC stock significantly increased under all the NT-Cs
669	systems, with an accumulation rate ranging from 0.70 to 1.07 Mg C ha ⁻¹ -yr ⁻¹ , while the SOC stock change
670	in CT-Cs was not significant and remain stable (Table 3).
671	Surprisingly, the response of soil N concentration to tillage systems differed from SOC. NT Cs crop
672	rotation systems (NT2-Cs and NT3-Cs) significantly increase soil N in the uppermost layer (0-5 cm) with
673	an average increase of 16% (0.28 g N kg ⁻¹ soil), whereas significant increases were observed by 10% in
674	5-10 cm and by 19% in 20-40 cm under NT2-Cs (Fig. 2F). Over a 10-year period, cassava monocropping
675	under NT systems (NT1-Cs) resulted in stable N concentration in the top 0-10 cm, but the concentration
676	significantly decreased below 10 cm by -10 to -25% from 10 to 100 cm depth (Fig. 2E). For CT-Cs, N
677	concentration remained stable throughout the soil profile, except a significant loss of -14% detected in
678	the 20–40 cm layer (Fig. 2E).
1	44

679	In the case of N stock, among the two NT Cs crop rotation systems (NT2 Cs and NT3 Cs), NT3 Cs
680	significantly increased N stock by 15% (0.1 Mg N ha ⁻¹) in the surface 0-5 cm, while significant increases
681	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
682	4). In the case of cassava monocropping, N stock in the NT1-Cs soil remained constant in the top 0-10
683	em, then significantly decreased from 10 to 100 cm from -0.3 to -0.5 Mg N ha ⁺ . In a similar trend to NT1-
684	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
685	Mg N ha ⁺ from 20 to 100 cm (Table 4).
686	From 2011 to 2021, considering 0-20 cm depth, all tillage and cropping systems did not alter N stocks
687	when compared to RV (Fig. 3F).
688	When considering the whole profile (0-100 cm), N stock in the NT Cs bi annual rotation systems did not
689	change with time. Regardless of tillage systems, long-term cassava monocropping resulted in significant
690	decreases of N with an annual depletion rate of -0.11 and -0.17 Mg N ha ⁺ yr ⁺ under CT-Cs and NT1-Cs,
691	respectively (Table 4).surprisingly, the response of soil TN concentration to tillage and cropping systems
692	differed from SOC (Fig. 2, with-Table S4 as duplicationin the supplementary as the duplication). The
693	positive ($p < 0.05$) effect on TN concentration was mainly observed on the surface layer under NT
694	systems. H: however, the significant ($p < 0.05$) decrease in TN concentration varied across tillage,
695	cropping systems and experiments observed below 20 cm (Fig. 2, with-Table S4-in the supplementary as
696	the duplication). In 2021, NTR systems (NTR1 and NTR2) significantly ($p < 0.05$) increased TN
697	concentration in the top 5 cm of MaiEx (NTR-Mz) and SoyEx (NTR-Sb) by 32% and 23%, respectively
698	(Figs. B2 and 2D), but decreased TN concentration significantly ($p < 0.05$) below 60 to 100 cm by -18 to
699	-21% and -10 to -25% in MaiEx and SoyEX, respectively (Figs. 2A and 2B). Under CasEx in 2021, the 45

500 soil TN concentration significantly ($p < 0.05$) increased by 16% in the top 0–5 cm under the N	<u>FR system</u>
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- 101 (average of NTR1-Cs and NTR2-Cs), while overall TN concentration remained neutral-stable below 5 cm,
- except for significant increases under NTR1-Cs by 10% and 19% in the 5-10 cm and 20-40 cm,
- 703 respectively (Fig. 2F).
- From 2011 to 2021, in the case of monocropping under NT systems in MaiEx and SoyEx, the significant
- (p < 0.05) increase in TN concentration by 44% and 25% under NTM-Mz and NTM-Sb, respectively
- (Figs 2B and 2C). However, the TN concentration was significantly (p < 0.05) decreased by -24% in the
- 40-60 cm under NTM-Mz and from -23 to -29% in the 60-100 cm depth under NTM-Sb (Figs. 2A and
- 2B). After 10-years of cassava monocropping under NT system (NTM-Cs), TN concentration did not
- $\frac{1000}{1000}$ change in the top 0–10 cm, but the concentration significantly (p < 0.05) decreased below 10–100 cm
- 710 <u>depth from -10 to -25% in 2021 (Fig. 2E).</u>
- In contrast to NT systems, after 10-years of conventional tillage-based monocropping of soybean (CTM-
- 512 Sb) and cassava (CTM-Cs), the soil TN concentration in 2021 remained constant across the whole profile,
- P13 except for a significant (p < 0.05) decrease loss of -14% in the 20–40 cm layer detected under CTM-Cs
- [14 (Fig. 2D and 2E), while in cassava monocropping under CT (CTM-Mz), soil TN concentration remained
- and 80–100 cm depths, respectively (Fig. 2A and 2B).



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Figure 2. TN concentration distribution across the soil profile (0–100 cm) of the treatments under different experiments in 2011 and 2021. CTM: monocropping under conventional tillage; NTM: monocropping under NT systems with no- till mulch-based cropping systems associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems under NT systems with no-till mulch-based cropping systems associated with different crop sequences as described in Table 1. A: MaiEx 2011 – TN concentration of the treatments in maize-based trial measured in 2011; B: MaiEx 2021 – TN concentration of the treatments in maize-based trial measured in 2021; C: SoyEx 2011 – TN concentration of the treatments in soybean-based trial measured in 2021; D: SoyEx 2021 – TN concentration of the treatments in soybean-based trial measured in 2021; E: CasEx 2011 – TN concentration of the treatments in cassava-based trial measured in 2011; and F: CasEx 2021 – TN concentration of the treatments in cassava-based trial measured in 2021. Treatment(s) in bold within the brackets indicate the gain and significant (p < 0.05) difference in concentrations between 2011 and 2021 in the same treatment at the same soil depth.

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717 <u>3.2.2 Total N stock</u>

- <u>Over the past decade, cultivating the main crops (maize, soybean, and cassava) under NTR systems</u>
- 719 (NTR1 and NTR2) significantly (p < 0.05) increased TN stock in the soil surface in all the experiments
- 20 in 2021. However, the response of TN stock below the surface layers to the NTR systems differed between
- the three experiments (Table 3, -and-Table S5-in-the supplementary). In the case of MaiEx (NTR1-Mz
- and NTR2-Mz), TN stock increased by 0.3 Mg N ha⁻¹ in the 0–5 cm; the stock remained neutral stable in
- the 5-40 cm, but then significantly (p < 0.05) decreased in the 40-100 cm depths between -0.25 and -0.40
- Mg N ha⁻¹ (Table 3). Similar to MaiEx, the soil TN stock in the soils under NTR systems of SoyEx (NTR1-
- Sb and NTR2-Sb) significantly increased by 0.25 Mg N ha⁻¹ in the 0–5 cm layer, remained constant in
- 726 the 5–60 cm, and then significantly decreased in the 60–100 cm with a rough amount of -0.02 Mg N ha⁻¹
- [727 (Table 3). In contrast to MaiEx and SoyEx, among the two NTR-Cs crop rotation system, NTR2-Cs
- 728 significantly (p < 0.05) increased TN stock by 0.10 Mg N ha⁻¹ in the surface 0–5 cm, whereas the
- significant increase in TN stock was detected in the 0-5, 5-10 and 20-40 cm by 0.10, 0.10 and 0.03 Mg

730	N ha-1, respectively, under NTR1-Cs. Unlike, MaiEx and SoyEx, there was no significant decrease in TN	
731	stock in the subsoil layers under the NTR-Cs (Table 3).	
732	When compared to 2011, TN stock in 2021 of MaiEx and SoyEx under the NTM system significantly (p	
733	<0.05) increased in the topsoil (0-5 cm) by 0.40 and 0.20 Mg N ha ⁻¹ under NTM-Mz and NTM-Sb.	
734	respectively. However, significant ($p < 0.05$) decreases in TN stock were detected in the subsoils under	
735	NTM-Mz by -0.40 Mg N ha ⁻¹ in the 40–60 cm and by -0.30 to -0.40 Mg N ha ⁻¹ in the 60–100 cm under	
736	NTM-Sb (Table 3). In the case of CasEx, TN stock in the NTM-Cs soil remained constant in the top 0-	
737	10 cm, then significantly decreased from -0.3 to -0.5 Mg N ha ⁻¹ in the 10–100 cm (Table 3).	
738	For the CTM of all the experiments, from 2011 to 2021, TN stock in the topsoil layers remained stable.	
739	whereas losses were observed in the layers below 20 cm. CTM-Sb significantly ($p < 0.05$) increased TN	
740	stock by 0.20 Mg N ha ⁻¹ in the 10–20 cm, then remained constant below 20 cm with a significant ($p <$	Formatted: Font: Italic, Complex Script Font: Italic
741	0.05) reduction by -0.20 Mg N ha ⁻¹ detected in the 60-80 cm (Table 3). In CTM-Mz, TN stock did not	
742	change in the 0-40 cm but significantly declined between -0.20 to -0.30 Mg N ha ⁻¹ from 40-100 cm	
743	(Table 3). In CTM-Cs soil, TN stock did not change in the top 0–20 cm but significantly decreased from	
744	-0.20 to -0.30 Mg N ha ⁻¹ in the 20–100 cm (Table 3).	
745	Measured in the whole profile (0-100 cm), over the past decade, the TN stock under NTR systems of all+	Formatted: Normal, Justified, Line spacing: Double
746	the experiments remained neutralstable (Table 3). Monocropping of soybean and cassava under NT	
747	systems (NTM-Sb and NTM-Cs) caused a significant ($p < 0.05$) reduction of TN stock at the annual	
748	depletion rate of -0.10 and -0.17 Mg N ha ⁻¹ yr ⁻¹ , respectively, while nearly a decade of upland rice	
749	monocropping then recent shifted to maize under NTM system (NTM-Mz) did not change TN stock	
750	(Table 3). In the case of monocropping of main crops under CT, the TN stock under CTM-Cs 49	

- significantly (p < 0.05) decreased at the rate of -0.11 Mg N ha⁻¹yr⁻¹. The TN stock of soil under CTM-Sb
- remained stable, while the CTM-Mz showed the depletion trend at the rate of -0.11 Mg N ha⁻¹ yr⁻¹
- 753 <u>although non-significant (Table 3).</u>

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Experiments*	Approximate	Cropping sy	stems^b						
	soil depth (cm)	CT	NT1	NT2	NT3	CT	NT1	NT2	NT3
		SOC stock i	n 2021 (Mg (7 ha⁻¹)		SOC stock c	hange rate 20)21-11 (Mg (ha⁻¹ yr⁻¹)
MaiEx	0-5	9.83 e	15.01 Aa	14.03 Aab	13.52 Ab	0.04	<mark>0.61</mark>	0.48	.0.4 4
	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
-	<mark>60-80</mark>	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	<u>-0.02</u>	_0.06
	0-100	86.92	90.13 A	92.90 A	88.27 A	0.17	1.30	,1.47	.86
SoyEx	<u>₽-5</u>	9.45 þ	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	<u>-0.02</u>	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	<u>p-5</u>	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

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

^aMaiEx: Maize-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment.

^bCT: 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 $P \le 0.05$ (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

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).05 (T	ukev's test). Positive val	ues of SOC :	stock change	rate indicate a	SOC accur	nulation: no	egative valu	ies indicate			
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le <u>3</u> 4. I	Mean <u>T</u> tota	N stock in 20)21 and $\underline{T}N$ s	stock change	rate between 2	2021 and 20	11.		•			
erime	Approxi Cropping systems ^b											
ntsª	mate soil depth	CTM	NT <mark>M</mark> 1	NT <u>R1</u> 2	NT <u>R2</u> 3	CT <u>M</u>	NTM1	NT <u>R1</u> 2	NT <mark>R2</mark> 3			
	(cm)	N stock in 2021 (Mg N ha ⁻¹)				N stock change rate 2021-11 (Mg N ha ⁻¹ yr ⁻¹)						
<u>MaiE</u>	0.50.5	0.79 (±0.04)	<u>1.30</u> A	<u>1.24 (±0.03)</u> <u>A</u>	<u>1.20</u> <u>A</u>	<u>-0.01</u>	0.04	0.03	0.03			
X	<u>0-5</u> 0-5	0.79	$\frac{(\pm 0.02)}{a}$	1.24 a	$-\frac{(\pm 0.04)}{1.20}$	<u>0.01</u>	(<u>±0.01)</u> 0.04	(<u>±0.00)</u> 0.03	(<u>±0.00)</u> 0.03			
	5 105 10	<u>0.79</u> h	<u>0.95</u> <u>A</u>	<u>1.04 (±0.02)</u>	1.06	<u>-0.01</u>	0.01	0.01	0.01			
A	<u>5-10</u> -10	<u>(±0.05)</u> 0.79 ₽	_ <u>(±0.01)</u> 0.95	1.04 #	<u>(±0.12)</u> 1.06 [#]	<u>0.01</u>	<u>(±0.00)</u> 0.01	<u>(±0.00)</u> 0.01	<u>(±0.01)</u> 0.01			
		1.55 g	1.44 B	1.87 (±0.23)	1.58 g	0.01	<u>-0.01</u>	0.03	0.00			
.	<u>10-20</u> 10-20	(±0.01)1.55 b	(±0.03)1.44 b	1.87	<u>(±0.12)</u> 1.58 b	<u>(±0.01)</u> 0.01	<u>(±0.01)</u> -	(±0.02)0.03	(±0.02)0.00			
		1.08	1.01	2.18	2.11	-0.02	-0.02	0.01	-0.02			
.	<u>20-40</u> 20-40	(±0.10) ^{1.98}	<u>(±0.06)</u> 1.91 ▲	<u>(±0.11)</u> 2.18 ▲	<u>(±0.05)2.11</u>	<u>(±0.02)</u> -	<u>(±0.00)</u> -	<u>(±0.02)0.01</u>	<u>(±0.01)</u> -			
						0.02 -0.02	0.02 -0.04	-0.03	-0.02			
	40-60 40-60	1.46 B	<u>1.33</u> <u>B</u>	<u>1.48 (±0.12)</u> <u>B</u>	<u>1.56 B</u>	(±0.01)-	(±0.00)-	(±0.01)-	(±0.01)-			
		(<u>±0.03)</u> 1.40 B	<u>(±0.11)</u> 1.33 В	<u>1.48</u> В	(<u>±0.05)</u> 1.30 B	0.02	0.04	0.03	0.03			
		1.24 B	1 49	1.25 (±0.04) B	136 B	<u>-0.02</u>	<u>-0.01</u>	<u>-0.04</u>	<u>-0.04</u>			
	<u>60-80</u> 60-80	(+0.09)1.24 B	(+0.13)1.49	<u>1.25 (20.01)</u> B	(10.02)1 <u>36</u> B	<u>(±0.01)</u> -	<u>(±0.01)</u> -	<u>(±0.01)</u> -	<u>(±0.00)</u> -			
		<u>(±0.07)</u> 1.21 B	(10.15)	1.25 D	<u>(10.02)</u> 1.00 D	0.02	0.01	0.04	0.04			
	00 100 20	1.00 P	1.22	1.21 (+0.02) P	117 P	-0.03	-0.02	-0.03	-0.04			
	<u>80-100</u> 80-	1.00 B	<u>1.22</u>	<u>1.21 (±0.05)</u> B	$\frac{1.17}{D}$	(±0.01)-	<u>(±0.01)</u> -	<u>(±0.01)</u> -	(±0.00)-			
	-100	(<u>±0.08)</u> 1.00 B	(±0.02)1.22	1.21 D	(<u>±0.03)</u> 1.1/ D	0.03	0.02	0.03	0.04			
				10.27	10.03	<u>-0.11</u>	-0.06	-0.03	-0.09			
	0-100 0-100	8.82	9.63	(±0.17) 10.2	(±0.30) 10.0	(±0.02)-	(±0.06) -	(±0.06)-	(±0.03)-			
•		<u>(±0.46)</u> 8.82 ▲	<u>(±0.49)</u> 9.63 ▲	7	2	0.11	0.06	0.03	0.09			
				· · ·		0.11	0.00	0.05	0.02			
SovE		0.82	1.15 <u>A</u>	1.13 (±0.03) A	1.20 <u>A</u>	<u>-0.01</u>	0.02	0.02	0.03			
x	<u>0-5</u> 0-5	(+0.03)0.82	(+0.01)1.15 A	1.13 A	(+0.06)1.20 A	<u>(±0.00)</u> -	(+0.00) 0.02	(+0.00)0.02	(+0.01)0.03			
.		<u></u>	a	a	a	0.01	<u></u>	<u></u>	<u></u>			
	5 105-10	1.07	0.87	0.99 (±0.02)	1.00	0.02	0.00	0.01	0.01			
	<u>3-103-10</u>	<u>(±0.22)</u> 1.07 ▲	<u>(±0.11)</u> 0.87 ▲	0.99	<u>(±0.03)</u> 1.00 ▲	<u>(±0.02)</u> 0.02	<u>(±0.01)</u> 0.00	<u>(±0.00)</u> 0.01	<u>(±0.00)</u> 0.01			
		1.75	1.52	1.74 (+0.00)	1.62	0.02	-0.01	0.01	<u>-0.01</u>			
	10-2010-20	<u>1.75</u> <u>A</u>	1.53	<u>1.74 (±0.09)</u>	1.62	0.02	(±0.00)-	0.01	(±0.01)-			
•	10 20 10 20	<u>(±0.12)</u> 1.75 A	<u>(±0.08)</u> 1.53 ▲	1.74	<u>(±0.02)</u> 1.62 ▲	<u>(±0.01)</u> 0.02	0.01	<u>(±0.01)</u> 0.01	0.01			
							-0.01	-0.01	-0.02			
		2.28	2.29	2.20 (±0.06)	2.14	0.02	-0.01	-0.01	(10.02)			
A	<u>20-40</u> 20-40	(±0.04) 2.28	(±0.12)2.29 ▲	2.20	<u>(±0.04)2.14</u> ▲	(±0.01)0.02	(±0.01)-	(±0.02)-	(±0.02)-			
			<u> </u>		<u> </u>	<u></u>	0.01	0.01	0.02			

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		<u>1.74</u>	<u>1.80</u> <u>A</u>	1.60 (±0.09)	<u>1.62</u>	0.00	<u>-0.02</u>	<u>-0.05</u>	<u>-0.02</u>	
A	<u>40-60</u> 40-60	<u>(±0.04)</u> 1.74 ▲	<u>(±0.05)</u> 1.80 A	1.60	<u>(±0.03)</u> 1.62	<u>(±0.01)</u> 0.00	<u>(±0.01)</u> -	<u>(±0.02)</u> -	<u>(±0.01)</u> -	Ľ
						-0.02	-0.03	-0.02	-0.02	
	co co 60 80	<u>1.44</u> <u>B</u>	<u>1.47</u> <u>B</u>	<u>1.44</u> <u>B</u>	<u>1.39</u> <u>B</u>	(+0.00)=	(+0.01)=	(+0.01)=	(+0.01)=	
A	<u>60-80</u> 00-80	(<u>±0.05)</u> 1.44 B	(<u>±0.08)</u> 1.47 B	<u>(±0.03)</u> 1.44 B	<u>(±0.02)</u> 1.39 B	0.02	0.03	0.02	0.02	~
						-0.01	-0.04	-0.04	-0.02	
	<u>80-100</u> 80-	<u>1.36</u>	<u>1.21</u> <u>B</u>	<u>1.18 (±0.04)</u> B	<u>1.26 (±0.01)</u> <u>B</u>	(+0.00)-	(+0.02)=	(+0.01)=	(+0.00)-	
	100	<u>(±0.04)</u> 1.36 ▲	<u>(±0.17)</u> 1.21 B		1.26 B	0.01	0.04	0.04	0.02	_
		10.45	10.31			0.01	-0.10	-0.06	-0.06	
	0-100 0-100	(±0.39) 10.4	(± 0.29) 10.3 $\frac{B}{D}$	<u>10.29 (±0.13)</u>	<u>10.23 (±0.12)</u>	0.02	(±0.03)-	(±0.03)-	(±0.03)-	
	<u></u>	5	+ B	10.29	10.23	<u>(±0.04)</u> 0.02	0.10	0.06	0.06	-
				А						
CasExCasE	A	0.76	0.84 b	0.95(+0.01) A	$\frac{A}{105(+0.04)}$	0.00	0.00	0.01	0.01	
<u>CasLA</u> CuSL	<u>0-5</u> 0-5	(+0.01)0.76 E	(+0.01)0.84	0.95 a	1.05 A	(+0.00)0.00	(+0.00000000000000000000000000000000000	(+0.00) 0.01	(+0.01)0.01	
T		<u>(</u> 017 0	<u>(=0.01)</u> 0101 e	biye u h	a	<u>(10007</u> 0100	<u>(</u> 0100	<u>(</u> 0.00)	<u>(</u> 0101_	
		0.82	0.88	0.89 (±0.02) A	0.91	0.00	0.00	0.01	0.00	
A	<u>5-10</u> 5-10	<u>(±0.02)</u> 0.82 ▲	<u>(±0.01)</u> 0.88 ▲	0.89 A	<u>(±0.01)</u> 0.91 •	<u>(±0.00)</u> 0.00	<u>(±0.00)</u> 0.00	<u>(±0.00)</u> 0.01	<u>(±0.00)</u> 0.00	_
		157 0	1.40 <u>B</u>	1.57 (+0.02) <u>A</u>	1.59 Å	<u>-0.01</u>	<u>-0.03</u>	0.01	0.00	\mathbb{Z}
	<u>10-20</u> 10-20	(10.03)157 h	(+0.07)1 <u>40</u> B	<u>1.57 (±0.02)</u> 1.57 a	(0.01)1.58 a	<u>(±0.01)</u> -	<u>(±0.01)</u> -	<u>(0.01</u>	<u>0.00</u>	- \
-		<u>(±0.05)</u> 1.57 0	<u>(±0.07)</u> 1.40 b	1.57 b	<u>(±0.01)</u> 1.50 a	0.01	0.03	(±0.00)0.01	<u>(±0.00)</u> 0.00	
		2.06 B	2.04 B	234 A	2 19	<u>-0.03</u>	<u>-0.03</u>	0.03	0.00	
A	<u>20-40</u> 20-40	(10.07)2.06 B	$(+0.10)^2 04 B$	$(+0.02)^2 34 A$	(+0.10)2 <u>19</u>	<u>(±0.01)</u> -	<u>(±0.01)</u> -		(+0.01)0.00	
		<u>(10.07)</u> 2.00 B	(<u>20.10)</u> 2.01 B	(20.02)2.01 11	(10.10)2.19	0.03	0.03	<u>(10.00)</u> 0100	<u>(=0.01)</u> 0.00	
		1.69 B	1.74 B	1.75 (±0.03)	1.74	-0.02	-0.03	0.01	-0.01	
A	<u>40-60</u> 40-60	(±0.05)1.69 B	(±0.05)1.74 B	1.75	(±0.06)1.74 ·	(±0.01)-	(±0.00)-	(±0.00)0.01	(±0.01)-	`
						0.02	0.03		0.01	
		<u>1.48</u> <u>B</u>	<u>1.51</u> B	1.54 (±0.02)	1.52 (±0.02)	<u>-0.02</u>	<u>-0.04</u>	0.00	<u>-0.02</u>	
▲	<u>60-80</u> 60-80	<u>(±0.10)</u> 1.48 B	<u>(±0.11)</u> 1.51 B	1.54	1.52	(±0.02)-	<u>(±0.01)</u> -	<u>(±0.00)</u> 0.00	(±0.01)-	- 1
						0.02	0.04 -0.05	0.01	0.02	
	<u>80-100</u> 80-	<u>1.32</u>	<u>1.29</u> <u>B</u>	1.34 (±0.05)	1.35 (±0.03)	(+0.01)	(+0.01)=	(+0.01)-	(+0.01)-	N.
A	100	<u>(±0.09)</u> 1.32 ▲	<u>(±0.11)</u> 1.29 B	1.34	1.35	0.02	0.05	0.01	0.02	7
						-0.11	-0.17	0.01	-0.03	
	0.1000-100	<u>9.70 B</u>	<u>9.70</u> <u>B</u>	<u>10.40 (±0.04)</u>	<u>10.34 (±0.25)</u>	(+0.06)-	(+0.07)-	0.06	(+0.01)-	Cale of the local division of the local divi
5	<u>0-100</u>	(±0.21)9.70 B	(±0.39)9.70 B	10.40	10.34	0.11	0.17	<u>(±0.02)</u> 0.06	0.03	
									0.05	1 101

^aMaiEx: Maize-based experiment; SoyEx: Soybean-based experiment; and CasEx: Cassava-based experiment. ^bCT<u>M</u>; monocropping under conventional tillage; <u>NTM</u>: monocropping under NT systems with no--till mulch-based cropping systems associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems <u>under NT systems with no-till mulch-based cropping systems</u> <u>NT</u>: 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*P <≤

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0.05 (Tukey's test). Values of TN stock change rates in bold indicate changes for a given treatment and depth between 2021 and 2011 are significantly different from 0 at $p \leq P \leq 0.05$ (Tukey's test). Positive values of TN stock change rate	 Formatted: Font: 12 pt, Font color: Auto, Complex Script Font: Italic
indicate a N accumulation; negative values indicate a N loss. <u>Values in the parentheses indicate standard errors (n=3)</u> .	Formatted: Font: 12 pt, Font color: Auto, Complex Script Font: Italic

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 3.3
 The iImpacts of tillage and cropping systems on organic C and N concenstorationks in size

 758
 fractions

759 3.3.1 C stock in size fractions

- ⁷⁶⁰ In this diachronic study, over the 10-year period, the stocks of C-POM and C-MAOM were significantly
- (p < 0.05) influenced by all the treatments. H; however, the effects varied across cropping systems and
- 762 the experiments (Fig. 4, with Table S6 and S7 in the supplementary as duplications).
- The data showed that C-POM stock in 2021 significantly (p < 0.05) increased in the surface layers (0–10)
- real the NT systems in MaiEx and SoyEx, but it was not the case in CasEx (Figs. 4B, 4D, and
- 765 <u>4F). The annual accumulation rates of C-POM stock in MaiEx and SoyEx were similar, with a ranged of</u>
- 766 approximately 0.15 and 0.04 Mg C ha⁻¹ yr⁻¹ under NTM system and 0.10 and 0.03 Mg C ha⁻¹ yr⁻¹ under
- NTR systems (average of NTR1 and NTR2) in the 0-5 and 5-10 cm, respectively. This suggested the
- consequence of the annual biomass inputs that were left on the soil surface under all the NT systems over
- the experimental period (Table 1). Although the significant increase in C-POM stock was also detected
- 170 <u>under CTM in the tilled layers (5–20 cm) in MaiEx and SoyEx, at the annual accumulation rates of only</u>
- 771 0.02 Mg C ha⁻¹ yr⁻¹ across the two soil depths (5–10 and 10–20 cm), which is relatively low when
- 772 compared with NT systems (Figs. 4B and 4D).
- In a similar trend to C-POM, C-MAOM stock increased significantly (p < 0.05) in the top soil depths
- 1774 under all the NT systems in MaiEx and SoyEx in 2021. The annual accumulation rates were similar
- between NTM-Mz and NTR-Mz, with a rate of 0.33 and 0.15 Mg C ha⁻¹ yr⁻¹ in the 0–5 and 5–10 cm,
- 776 respectively (Fig. 4B). In SoyEx, all the NT systems exhibited the trend of C-MAOM stock accumulation
- in the deeper layers (to 20 cm) than MaiEx, with approximate annual accumulation rates of 0.20, 0.15,

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and 0.10 Mg C ha ⁻¹ yr ⁻¹ in the $0-5$, $5-10$, and $10-20$ cm, respectively (Fig. 4D). In CasEx, despite	te the fact
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that the C-POM stock remained constant over the past decade, the C-MAOC stock significantly (p < 0.05)

- 780 increased down to 40 cm by all the NT systems in 2021, with similar accumulation rates from 0.09 to
- 781 $0.26 \text{ Mg C h}^{-1} \text{ yr}^{-1}$ in the 0–40 cm depths (Fig 4F).
- 782 3.5 Under CTM in 2021, an increase in C-MAOM stock was observed in the tilled layers across all*
- experiments (Figs. 4B, 4D, and 4F). Specifically, in the MaiEx experiment, significant differences (p < 1
- 784 0.05) of C-MAOM stock between 2011 and 2021 were found in the 5–10 cm and 10–20 cm layers, with
- annual accumulation rates of 0.10 and 0.23 Mg C ha⁻¹ yr⁻¹, respectively (Fig. 4B). In the case of SoyEx,
- a significant increase in C-MAOM stock was only detected in the 10-20 cm layer, with an annual
- accumulation rate of 0.11 Mg C ha⁻¹ yr⁻¹ (Fig. 4D). Meanwhile, in the CasEx experiment, the C-MAOM
- stock showed a significant annual increase at a rate of 0.05 Mg C ha⁻¹ yr⁻¹ across the topsoil 0–20 cm
- 789 <u>(Fig. 4F).</u>

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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

3.5.1 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 lavers (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 em depths under CT-Mz and NT1-Mz, respectively (Fig. 5A and 5B).

3.5.1 Sovbean-based experiment

<mark>Under this diachronic analysis, the concentration of C and N in the POM fraction showed similar⁴ patterns and magnitudes as in MaiEx (Fig. 4 and 5). C POM in the top 0–20 cm was influenced by</mark>

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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 em, 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).

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).

3.5.2 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 erop 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 Formatted: Caption

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(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). 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).



Figure 4. Amount of Carbon stock in mineral-associated and particulate organic matter (MAOM_and POM) and particulate organic matter (POM) fractions across the whole profile (01–100 cm) in 2011 and 2021 under different treatments and experimentseropping systems. CTM: monocropping under conventional tillage; NTM: monocropping under NT systems with no-till mulch-based cropping systems

Formatted: Font: Bold, Complex Script Font: Not Bold Formatted: Font: Bold, Complex Script Font: Not Bold associated with different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems under NT systems with no-till mulch-based cropping systems associated with cropping systems 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. The uUppercase letters on the bars and lowercase letters in front of the bars indicate a significant difference (Tukey's test; <u>pP</u> < 0.05) in C <u>estonckentration</u> in MAOM and POM, respectively, between 2011 and 2021 for the same treatment and soil depth.

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790 3.3.2 N stock in size fractions

791 Over the past decade (2011–2021), tillage and cropping systems had varying effects on the stocks of N-

	66	
812	5 cm to subsoil layers, but this was inconsistent between the two NTR systems (NTR1 and NTR2) and	
811	and 5C). Under NTR-Mz and NTR-Sb, the significant decrease of N-MAOM stock was detected below	
810	significantly ($p < 0.05$) below 40 cm with the rate ranging from -0.036 to -0.063 Mg N ha ⁻¹ yr ⁻¹ (Figs. 5A)	
809	remained unchanging under NTM system (i.e., NTM-Mz and NTM-Sb) from 0 to 40 cm but declined	
808	decreased it with varying soil depths and experiments (Fig. 5). In MaiEx and SoyEx, N-MAOM stock	
807	Surprisingly, from 2011 to 2021, none of the tillage or cropping systems increased N-MAOM stock, but	
806	<u>60 cm (Fig. 5E and 5F).</u>	
805	CTM-Cs soil remained stable in the top 20 cm, but significantly declined by -0.01 Mg N ha ⁻¹ from 20 to	
804	Mz) did not change the N-POM stock across the soil profile (Fig. 5B), whereas the N-POM stock under	
803	Monocropping of upland rice over a decade and recent shifted to maize under conventional tillage (CTM-	
802	layers, but the significant depletion ($p < 0.05$) at a similar amount was observed below 40 cm (Fig. 5D).	
801	POM stock in the tilled layers (5-10 and 10-20 cm) with an amount of 0.01 Mg N ha ⁻¹ across the two	
800	In 2021, monocropping of soybean under conventional tillage (CTM-Sb) significantly accumulated N-	
799	top soils, but NTM-Cs and NTR-Cs systems significantly ($p < 0.05$) depleted it below 20 cm (Fig 5F).	(
798	and 5D). In contrast to MaiEx and SoyEx, in CasEx, none of NT systems increased N-POM stock in the	
797	under a NTR system, in particular under NTR2-Mz below 40 cm and NTR2-Sb below 60 cm (Figs. 5B	
796	remained constant under all the NT systems in both experiments, except for the depletion trend found	
795	ha ⁻¹ in the 0-5 and 5-10 cm, respectively (Figs. 5B and 5D). Below 10 cm, overall, N-POM stock	
794	(0-10 cm) under all the NT systems in MaiEx and SoyEx, with similar amounts of 0.10 and 0.01 Mg N	
793	supplementary as duplications). In 2021, N-POM stock increased significantly ($p < 0.05$) in the topsoil	
792	POM and N-MAOM across soil depths and the experiments (Fig. 5, with-Tables S8 and S9-in-the	

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813	soil depths, with	n depletion rate	s ranging from	-0.023 Mg N ha-1	yr ⁻¹ in the near soil	surface to -0.140 Mg
		-			-	

- N ha⁻¹ yr⁻¹ in the bottom of the soil profile (Figs. 5A and 5C). In CasEx, the N-MAOM stock in the surface
- 815 layer (0-5 cm) did not change under all the NT systems (NTM-Cs, NTR1-Cs, and NTR2-Cs), but
- 816 decreased significantly (p < 0.05) below 5 cm with the annual depletion rates ranging from -0.009 to Mg
- 817 N ha⁻¹ yr⁻¹ in the 5 cm to -0.111 Mg N ha⁻¹ yr⁻¹ in the subsoil profile (Fig. 5E and 5F).
- 818 In 2021, the N-MAOM stock of the CTM-Mz soil remained steady at the 0-40 cm, whereas depletion-
- 819 was detected from 40–100 cm at rates ranging from -0.032 to -0.058 Mg N ha-1 yr-1 (Figs. 5A and 5B).
- 20 CTM-Sb did not preserve N-MAOM stock even in tilled layers over the past ten years, but depleted it
- significantly below 5 cm to subsoil depths at rates of -0.016 to -0.073 Mg N ha⁻¹ yr⁻¹ (Figs. 5C and 5D),
- 822 while a significant decrease in N-MAOM stock was observed throughout the soil profile (0-100 cm) with
- 823 <u>a depletion of -0.013 to -0.081 Mg N ha⁻¹ yr⁻¹ (Figs. 5E and 5F).</u>

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	Figure 5. <u>Amount of TN stock</u> in mineral-associated organic matter (MAOM) and particulate organic matter (MAOM and POM) fractions across the whole profile (01–100 cm) in 2011 and 2021 under	\langle	Formatted: Font: 12 pt, Complex Script Font: 12 pt, Not Bold
	different treatments and experiments cropping systems. <u>CTM: monocropping under conventional tillage;</u> NTM: monocropping under NT systems with no- till mulch-based cropping systems associated with		Formatted: Font: Bold, Complex Script Font: Not Bold
	different crop sequences, and NTR1 and NTR2 refer to bi-annual crop rotational systems under NT		
	systems with no-till mulch-based cropping systems associated with cropping systems as 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. <u>The uUppercase letters on the bars and lowercase letters</u>	C	
	in front of the bars indicate a significant difference (Tukey's test; $\underline{pP} < 0.05$) in <u>TN steonckentration</u> in MAOM and POM, respectively, between 2011 and 2021 for the same treatment and soil depth.		Formatted: Font: Italic, Complex Script Font: Not Italic
824	4 Discussion	(Formatted: Font color: Auto
825	4.1 Change in SOC stock		
826	Despite the varied contrasted effects among the NT systems and the experiments, our This_study showed		
827	that quantified the impacts adopting NT systems with the use of cover crops and high biomass C inputs		
828	in the long-term significantly of cropping systems on changes-increased SOC-and N stocks and their		
829	fractions down to 100 cm depth in three long term annual crop production experiments. Over 10 years,		
830	NT systems modified the SOC stock and its vertical distribution (Table 3).	(Formatted: Font color: Auto
831	Several studies reported that long-term NT adoption accumulated SOC stock only on the surface soils,		
832	but the stock did not differ from CT when considering the whole soil profile (Blanco-Canqui and Lal,	(Formatted: Font color: Auto
833	2008; Luo et al., 2010; Blanco-Canqui et al., 2011; Du et al., 2017; Xiao et al., 2020). For example, a		
834	recent meta-analysis from 86 studies covering a range of crop productions across the world, (Xiao et al.,		
835	(2020) found that NT systems significantly accumulated the SOC stock only in the top 0–5 cm, and no		
836	significant change was found below 5 cm. Across climatic conditions, soil types, and various cropping		
837	systems in China, based on 95 comparisons between NT and CT, adopting NT led to increase in SOC		
838	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). 69	(Formatted: Font color: Auto

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 843 844 systems (Paustian et al., 1997; Six et al., 2002; Bayer et al., 2006; Ogle et al., 2012; Virto et al., 2012). 845 Soils in the tropical climate require diversified and large amounts of C inputs for NT viability due to fast 846 residue decomposition and difficulty in maintaining soil cover (Séguy et al., 2006; Castro et al., 2015). 847 In the same experiments as in our study, Hok et al. (2015) reported that NT systems with diverse crop 848 species significantly accumulated SOC at the surface 0-5 cm after 4 years of NT adoption. Our study 849 Although there is variability in SOC stock accumulation and its vertical distribution among the three NT 850 systems, our results revealed that NT systems significantly increased SOC stock, although there wais 851 variability among the NT systems, and across the three experiments in the accumulation rates in the 852 subsoil layers (Table 2) 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⁺ yr⁺ in 0 20 cm under MaiEx, and from 0.69 to 0.86 Mg C ha⁺ yr⁺ 853 854 in 0-40 cm under CasEx. Considering the cumulative SOC stock, our results revealed that all the NT 855 systems significantly (pP > 0.05) increased cumulative SOC stock across the whole soil profile in MaiEx 856 and CasEx. In SoyEx, significant increase in cumulative SOC stock was limited to the top 0-20 cm under NTM-Sb-monocropping, whereas NT<u>R-Sb</u> crop rotation systems had significantly accumulating SOC 857 858 stock from 0 to 80 cm depths (Table 5) (the cumulative SOC stock in 2011 is presented in Table S109 in 859 the supplementary materials).

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881	and diverse quality of C inputs above- but also below-ground that were retained to the soils. In our
882	experiments, the annual biomass C inputs retained in the NT soils ranged from 3.61 to 6.07 Mg ha ⁻¹ yr ⁻¹ ,
883	versus 1.36 to 2.20 Mg ha ⁻¹ yr ⁻¹ under CTM (Table 1). In a elayed-clayey Oxisol of Brazil, a 16-year-old
884	experiment revealed that NT was more effective than CT at converting biomass-C inputs into SOC, with
885	a C conversion ratio in 0-40 cm depth of 0.35 compared to 0.07 under NT and CT, respectively (Sá et al.,
886	2014). In addition, integration of cover crops into the crop production system led to a significant increase
887	in SOC. From the observation of 139 plots at 37 sites from the tropics and temperate zone and diverse
888	soil types, Poeplau and Don (2015) reported that the use of cover crops led to an average SOC
889	accumulation rate of 0.32 Mg C ha ⁻¹ yr ⁻¹ at 22 cm depth. Association of tropical legume cover crops in
890	maize production led to increased SOC stock in the surface as well as the whole soil profile. Diekow et
891	al. (2005) found that the SOC accumulation rate in the legume-based cropping systems was 0.83 and 1.42
892	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
893	Brazilian Acrisol. From a 30-year-old experiment in a Brazilian Acrisol, legume cover crops were twice
894	as effective in storing C as mineral N fertilization, with 1 kg of residue C input being transformed to 0.15
895	kg of SOC (Veloso et al., 2018).
896	Considering the challenges faced by smallholder farmers in Cambodia with low financial resources and/or
897	high level of indebtedness, the main strategy should focus on enhancing nutrients cycling through
898	continuous biomass-C inputs under no-till cropping systems plus a combination of actions to reduce
899	nutrient removal from cassava fields through the non-removal of leaves and of a proportion of stalks, that
900	may also help to reduce the impact of nutrients deficiency. In addition, the tolerance of cassava to acidic
901	soil, its ability to grow on depleted and degraded soils related to the occurrence and synergistic effects of

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902	arbuscular mycorrhizal fungi (Howeler et al., 1982; Howeler and Sieverding, 1983), and plant growth-
903	promoting rhizobacteria (PGPR) (Balota et al., 1999), its nutrient recycling ability through leaf litter and
904	when the stalks are not used as planting materials and kept into the field, could be used to advance
905	inimprove soil and cropping system sustainability (Fermont et al., 2008). This possible use by farmers of
906	cassava cropping systems as a strategy for regenerating soil fertility was also emphasized by Saïdou et
907	al., (2004) and Adjei-Nsiah et al., (2007) in Benin and Ghana, respectively. During the decomposition
908	process, microbial communities use the rapidly decomposable materials as energy sources, while the
909	recalcitrance and other labile compounds materials act as the glue to bind soil mineral particles together
910	(Witzgall et al., 2021). This process is a pathway for the formation of soil micro aggregates (Bot and
911	Benites, 2005). The continuous supply of biomass C inputs to the soil associated with microbial
912	decomposition without soil mechanical disturbance creates a favourable environment for the emergence
913	of soil macroaggregates (Crews and Rumsey, 2017). Organic carbon inside soil aggregates is physically
914	protected from microbial oxidation as well as strongly associated with the organo minerals, leading to
915	SOC stabilization over time (Powlson et al., 1987; Lützow et al., 2006). In the same experiments as in
916	our study but after 3 years of NT adoption, Hok et al. (2021) reported that soil aggregation was one of the
917	main stabilization mechanisms, providing physical protection to the newly derived C into the soil
918	microaggregates protected by macroaggregates. From our knowledge of the literature, the high SOC
919	accumulation rate recorded under cassava-based NT cropping systems is relatively unique and, in addition
920	to the residues of cover crops and maize under the biannual cropping system, the nature of the cassava
921	residues that was retained into the field with high cellulose and lignin contents (Veiga et al., 2016) may
922	explain this result. Considering the challenges faced by smallholder farmers in Cambodia with low
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923	financial resources and/or high level of indebtedness, the main strategy should focus on enhancing
924	nutrients cycling through continuous biomass C inputs under no till cropping systems plus a combination
925	of actions to reduce nutrient removal from cassava fields through the non-removal of leaves and of a
926	proportion of stalks, that may also help to reduce the impact of nutrients deficiency. In addition, the
927	tolerance of cassava to acidic soil, its ability to grow on depleted and degraded soils related to the
928	occurrence and synergistic effects of arbuscular mycorrhizal fungi (Howeler et al., 1982; Howeler and
929	Sieverding, 1983), and plant growth-promoting rhizobacteria (PGPR) (Balota et al., 1999), its nutrient
930	recycling ability through leaf litter and when the stalks are not used as planting materials and kept into
931	the field, could be used to advance in soil and cropping system sustainability (Fermont et al., 2008). This
932	possible use by farmers of cassava cropping systems as a strategy for regenerating soil fertility was also
933	emphasized by Saïdou et al., (2004) and Adjei-Nsiah et al., (2007) in Benin and Ghana, respectively.
934	Long-term NT adoption has been shown to significantly improve soil structure, soil porosity and pore
935	connectivity (Cooper et al., 2021) contributing to the improvement of water infiltration, gas exchanges
936	and microbial activities, and roots development to deeper soil profile (Rosolem et al., 2016). In addition,
937	aerobic condition of soil aggregates would enhance SOC stability in unsaturated soils (Zhang et al., 2021).
938	Sisti et al. (2004) showed that increased C accumulation in NT soil below 30 cm depth could be explained
939	by greater root density when compared with CT. Another possibility is that organic residues from upper
940	layers were transported downward by soil meso- and macro-fauna organisms, which could have been
941	favoured by better environmental conditions provided by the continuous C flow and soil structure

942 enhancement under NT systems (Lavelle et al., 2016).

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943	In our study, the SOC stock in the whole soil profile (0–100 cm) under CTM and for the three experiments
944	remained constantstable, which could be attributed to the fully retained crop residues (i.e., mungbean,
945	rice, and maize) in MaiEx and (i.e., sesame and soybean) in SoyEx and partially retained cassava's fallen
946	leaves and stalks in CasEx (Table 1), indicating that it reached a new SOC equilibrium-stage. The high
947	clay content has also contributed to the stabilisation of the MAOC that accounted, in 2021, for 97.2% of
948	the carbon concentrationsstock along the soil profile.
949	Under a synchronic approach, considering CTM as the reference, the SOC stock change rates in 2021
950	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,
951	SoyEx, and CasEx, respectively (Fig. 6). When compared with the diachronic approach, this corresponds
952	to an underestimation of 146 to 536%, 51 to 347%, and 51 to 997% in MaiEx, SoyEx, and CasEx,
953	respectively (Table 3). In Brazil, from on-farm assessments of the SOC dynamics under long-term NT
954	systems in tropical heavy clayedy soils Neto et (al., 2010) and Junior et al., (2013) reported that
955	synchronic approach led to the biased in the annual SOC accumulation rates under NT systems when
956	compared with diachronic approach. The main factors associated with the errors could be the underlying
957	heterogeneities of the soil conditions prior to the conversion to NT systems that are hard to capture despite
958	all the steps of the methodologically precautious measurements being implemented properly (Neto et al.,
959	2010; Junior et al., 2013). Our findings clearly emphasize the importance of the diachronic approach in
960	accurately estimating the effects of long-term CA and NT systems on SOC storage, as well as providing
961	a proper interpretation of their roles in climate change mitigation through SOC sequestration.

Table 5. Cumulative SOC stock in 2021 and cumulative SOC stock change 2021 2011. 962

periments*	Approximate	e Croppir	ig system :	ş ^b -						Bottom: 1.5 cm, Width: 21 cm, Height: 24 cm
	soil depth (cm)	CT	NT1	NT2	NT3	CT	NT1	NT2	NT3	Formatted: Font color: Auto
	(011)	Cumulative SOC stock 2021 ha ⁻¹)		21 (Mg C	Cumulative SOC stock change 2021-11 (Mg C ha ⁻¹)			-11 (Mg C		
MaiEx	0-10	19.98 Ab	25.93 Aa	25.18 - Aa	24.40 - Aa	1.24	8.30	7.06	6.64	 Formatted: Font color: Auto
A	0-20	38.26 A	4 2.24 A	4 2.26 A	4 0.72 A	3.53	9.37	9.56	8.49	 Formatted: Font color: Auto
A	0-40	58.16 A	60.85 A	62.55 A	59.37 A	4.61	10.94	14.03	<u>9.22</u>	 Formatted: Font color: Auto
A	0-60	70.74	73.03 A	75.29 A	71.68 A	4.19	11.76	15.07	9.40	Formatted: Font color: Auto
A	0-80	79.75	82.61 A	84.93 A	81.01 A	3.01	12.45	14.89	9.21	 Formatted, Font color, Auto
A	0-100	86.92	90.13 A	92.90 A	88.27 A	1.75	12.97	14.71	<u>8.59</u>	
A										Formatted: Font color: Auto
SovEx	0-10	19.41b	22.91	24.19	24.38					Formatted: Font color: Auto
	0.00	27.76	Aab	- Aa	Aa	0.46	4.00	4 .96	5.61	Formatted: Font color: Auto
A	0-20	3/./0	<u>39.70 A</u>	41.46 A	41.81 A	1.52	3.76	6.61	6.37	 Formatted: Font color: Auto
A	0-40	60.10	62.35	60.72 A	61.50 A	3.45	5.43	8.55	<u>8.19</u>	Formatted: Font color: Auto
A	0.80	75.30 86.45	78.21 00.16	+3.12 A	74.39 A	<u>4.82</u>	6.46	9.81	9.88	Formatted: Font color: Auto
A	0-30 0-100	<u>95.22</u>	<u>90.10</u>	90.23	91.15	<u> </u>	7.03	9.96	10.02	 Formatted: Font color: Auto
	0 100	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		,0120	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	4.70	0.55		10.05	 Formatted: Font color: Auto
CocEv	0.10	17.41	19.00	20.50	21.43					Formatted: Font color: Auto
Casex	0-10	Ac	Abe	Aab	Aab	1.46	3.57	3.09	3.86	 Formatted: Font color: Auto
_	0-20		35.66 Asb	37.13 Aab	37.66 	2 30	6.04	3.08	5 60	
	0.40	52 74	54 50 A	57 00 A	56.03 A	1.45	0.04	6.00	0.57	Formatted: Font color: Auto
A	0.60	65.96	67.02 A	71.94 A	60.02 A	1.43	8.48	0.05		Formatted: Font color: Auto
^	0.80	76.07	78 71 A	71.04 A	80.10 A	1.00	9.72	8.05	9.82	Formatted: Font color: Auto
A	0_100	86.44	87.42 A	91.07 A	88.01 A	<u>1.20</u>	10.68	7.93	<u>10.25</u>	Formatted: Font color: Auto
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*MaiEx: Maize based experiment; SoyEx: Soybean based experiment; and CasEx: Cassava based experiment.

^bCT: 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 $P \le 0.05$ (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 $P \le 0.05$ (Tukey's test).

963	In our study, the SOC stock in the whole soil profile (0-100 cm) under CT and for the three experiments		
964	remained constant, which could be attributed to the fully retained crop residues (i.e., mungbean, rice, and		
965	maize) in MaiEx and (i.e., sesame and soybean) in SoyEx and partially retained cassava's fallen leaves		
966	and stalks in CasEx (Table 1), indicating that it reached a new SOC equilibrium stage. The high clay		Formatted: Font color: Auto
967	content has also contributed to the stabilisation of the MAOC that accounted, in 2021, for 97.2% of the		
968	earbon concentrations along the soil profile.		
969	Under a synchronic approach, considering CT as the reference, the SOC stock change rates in 2021 under		
970	NT systems ranged from 0.13 to 0.60, 0.50 to 0.43, and 0.10 to 0.46 Mg C hat yr in MaiEx, SoyEx,		Formatted: Font color: Auto
971	and CasEx, respectively (Fig. 6). When compared with the dischronic approach, this corresponds to an		Formatted: Font color: Auto
972	underestimation of 146 to 536%, 51 to 347%, and 51 to 997% in MaiEx, SoyEx, and CasEx, respectively		Formatted: Font color: Auto
973	(Table 3). In Brazil, from on-farm assessments of the SOC dynamics under long-term NT systems in		Formatted: Font color: Auto
974	tropical heavy clayed soils Neto et (al., 2010) and Junior et al., (2013) reported that synchronic approach		Formatted: Font color: Auto
975	led to the bias in the annual SOC accumulation rates under NT systems when compared with diachronic		Formatted: Font color: Auto
976	approach. The main factors associated with the errors could be the underlying heterogeneities of the soil		
977	conditions prior to the conversion to NT systems that are hard to capture despite all the steps of the		
978	methodologically precautious measurements being implemented properly (Neto et al., 2010; Junior et al.,	_	Formatted: Font color: Auto
979	2013). Our findings clearly emphasize the importance of the diachronic approach in accurately estimating		





⁹⁸¹ 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 (n = 3; error bars = SE). A: MaiEx (maize-based trial); B: SoyEx (soybean-based trial); and C: CasEx (cassava-based experiments). CAM: monocropping under conservation agriculture with no-till mulch-based cropping systems associated with different crop sequences, and CAR1 and CAR2 refer to bi-annual crop rotational systems under conservation agriculture with no-till mulch-based cropping systems associated with different crop sequences described in Table 1. The stock change rates under diachronic were calculated by subtracting the stock of the same treatment in 2021 from the stock in 2011 and dividing by the number of years between the 1st and 2nd samplings (10 years), while the stock change rates of CA systems in 2021 under synchronic were calculated by subtracting the stock of each CA treatment from the stock of CTM in 2021, considered the control, and dividing by the number of years between the 1st and 2nd

Formatted: Font: Bold, Complex Script Font: Not Bold Formatted: Font: Bold, Complex Script Font: Not Bold samplings (10 years). (*) indicates a significant difference (Tukey's test; p < 0.05) in SOC stock between 2011 and 2021. Positive values indicate SOC stock accumulation; negative values indicate SOC loss.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).

982 4.2 Change in N stock

983 In addition to increasing SOC stock in the surface and the whole soil profile. Diekow et al. (2005) found 984 that soil-total TN stock was significantly increased by an average of 27% inat the surface (0-17.5 cm) 985 and by 6% in the whole profile (0-107.5 cm) after 17 years of NT maize and -with the use of tropical 986 legume intercroppingeover crops and N fertilization compared <u>in comparison to</u> with its original state 987 under native grassland as a reference ofin a Brazilian Acrisol. Sá et al., (2014) reported a strong 988 positive ignificant relationship-correlation ($R^2 = 0.89$, P < 0.0002) between the soil N and SOC stock 989 accumulation. E; each unit of N stock accumulation contributed to the sequestration of 10.2 Mg C ha⁻¹ at 990 the top 0-10 cm under long-term (16-year) continuous NT maize-based production of Brazilian's Oxisol. 991 However, the diachronic assessment in our study showed that soil T-N stock under NT systems 992 significantly increased only in the topsoil (0-5 cm) in MaiEx and SoyEx, while the stock remained stable 993 in CasEx (Table 3). The significant decline of TN stock under NT systems, although with variability 994 across the NT systems and the experiments, was detected below 20 cm. When considering the whole 995 profile (0–100 cm), significant depletion of N stock was observed under the NT monocropping systems, 996 with a loss rate at -0.10 and -0.17 Mg N ha⁻¹ yr₁⁻¹ in SoyEx (NTM1-Sb) and CasEx (NTM1-Cs), 997 respectively. Under NT crop rotation systems, despite non-significant, TN 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). 998

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999	The depletion of TN stock under NT was reported from short- (Wuaden et al., 2020) to longer-term NT	_	Formatted: Font color: Au	ıto
1000	adoption (Delgado, 2023). From a short-term (5-year) conversion of native grassland to cropland under		Formatted: Font color: Au	ıto
1001	NT adoption with a double cropping with maize as a cash crop followed by black oat as a cover crop in			
1002	Brazil's Rhodic Nitosol, NT soils had significant losses of soil total N in comparison with the original			
1003	stocks under grassland throughout the soil profile, with the exception of the $0-5$ and $10-20$ cm soil layers.			
1004	Considering the whole profile (0–60 cm), soil total N was depleted by <u>1.7 Mg N ha⁻¹</u> , equivalent to an			
1005	annual loss rate of -0.34 Mg N ha ⁻¹ yr ⁻¹ after 5 years of grassland conversion to NT (Wuaden et al., 2020).		Formatted: Font color: Au	ıto
1006	Results from a 12-year experiment in the US (0-120 cm depth) in an irrigated NT continuous maize			
1007	rotation where mineral N were applied at different rates indicated that even NT could potentially have			
1008	significant net N loss with an average loss of $=15 \text{ kg N} \text{ ha}^{-1} \text{ yr}^{-1}$ at the top 30 cm of soil regardless of N			
1009	application rate (Delgado, 2023).		Formatted: Font color: Au	ıto
1010	In our study, it is a rather surprising finding to observe an increase in SOC and a simultaneous soil $\underline{T}N$			
1011	depletion. Associating legume cover crops in the cropping system did not enhance soil N through			
1012	biological N fixation (Rosolem et al., 2016). In general, N is an important factor contributing to SOC		Formatted: Font color: Au	ito
1013	storage (De Vries, 2014; Kirkby et al., 2014). Nutrient reserves are among other factors that determine		Formatted: Font color: Au	ıto
1014	soil C storage capacity (Lal, 2018). Therefore, more studies on nutrient availability and their		Formatted: Font color: Au	ıto
1015	stoichiometry relationship including in deeper layers (>100 cm), on the N use efficiency and N cycling			
1016	are needed to understand the driving mechanisms of the N dynamics under these NT systems.			
1017	Nitrogen uptake and/or N priming effects from the cover crops, among other factors, could possibly have			
1018	resulted in N loss in our study. Priming effects are short-term changes in the turnover of soil N caused by			
1019	the addition of organic or mineral fertilizer, the mechanical treatment of soil, its drying and rewetting 81			

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1020	(Kuzyakov et al., 2000), and the exudation of organic substances in the rhizosphere by living plants
1021	(Kuzyakov, 2002). These effects can occur immediately or very shortly after the addition of a specific
1022	substance to the soil and are larger in soils rich in C and N than those in poor soils (Kuzyakov et al.,
1023	2000). In our experiments, under CA systems, the soils are year-round protected by the cover crops
1024	established through association or succession with the main crops (maize, soybean, and cassava) and
1025	continue to grow after the main crop harvest. Several species of drought-tolerant and fast-growing cover
1026	crops (stylo, brachiaria, cowpea, sorghum, pearl millet, and sumnhemp), which are commonly used in our
1027	experiments as a single or mixture (Table 1), are good examples of remaining green throughout the dry
1028	season with root exudates that may have enhanced the priming effect. In addition, the symbiosis
1029	relationship between the cover crops and rhizobia during the dry season could also be low due to low soil
1030	moisture content, therefore resulting in high N uptake from the soil by those cover crops. Their drought-
1031	tolerant characteristics allow these species to cross the dry season, even with little or no rain for more
1032	than 4 months in the dry season. Their fast-growing characteristics, along with the species diversity,
1033	produced a large amount of biomass annually and were retained in the soil at the termination of the
1034	cultivation of the main crops (Table 1), which may create conditions for the N uptake or N priming effects
1035	to happen. Therefore, the mMeasurement of N content and the estimation of biological nitrogen fixation
1036	by the legume cover crops using the ${}^{15}N^{45}$ isotopic technique should be conducted to better understand N
1037	dynamics in the different systems.explain the N uptake or N priming effects.
1038	To date, few studies on the impact of no-till systems on N gains or N losses, N use efficiency and changes
1039	in soil N have been conducted, and the results vary depending on the period of NT adoption and sampling
1040	depths (Congreves et al., 2017; Delgado, 2023). Further research is needed to understand the driving 82

1041 mechanism of the N dynamics under NT systems by considering deeper layers (>100 cm) for making 1042 informed decisions regarding sustainable soil fertility management and crop production systems. Positive 1043 accumulation rates of SOC stock, recorded under NT systems, could not be sustained on the long-term as 1044 the depletion of the <u>TN</u> stock may lead to nutrient scarcity of other nutrients (P, S, Ca²⁺ and Mg²⁺) that is 1045 the driving force limiting SOC accumulation. Further analysis is needed to assess the coming changes in 1046 SOC and N stocks along with the content of nutrients and its potential impact on the rate of SOC 1047 accumulation or depletion (Kirkby et al., 2013).



4.3 SOC and N stocks recovery after land use change

1049 Conversion of native vegetation to cropland under conventional plough based tillage depletes SOC (Sisti 1050 et al., 2004; Sá et al., 2015; Wuaden et al., 2020) due to soil structure disruption by the mechanical 1051 disturbance, low C inputs, and accelerates the SOC mineralisation rate by exposing SOC that was 1052 encapsulated inside soil aggregates to microbial oxidation (Balesdent et al., 2000). Even if this 1053 comparison with RV was restricted to 0.20 cm depth, the present study showed that NT systems can 1054 restore SOC stock that was lost during crop production under conventional tillage after the conversion of 1055 native vegetation to cropland and before the experiments' establishment. At 0 20 cm depth, the annual 1056 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 1057 ⁴ yr⁴ under MaiEx, SoyEx and CasEx, respectively. This could be attributed to the long term NT systems 1058 adoption with multiple crop species through cash crop rotation and cover crop association producing high 1059 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-1060

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1062	vegetation in Brazil. Sá et al., (2014) reported the recovery of SOC stock under NT to the reference
1063	vegetation an accumulation rate of 0.84 Mg C ha ⁺ yr ⁺ at 0–20 cm after 16 years of continuous NT with
1064	an average annual biomass C input of 4.40 Mg ha ⁺ yr ⁺ .
1065	Noticeably, SOC stock under CT was significantly lower than RV in 2011 in all the three experiments,
1066	but SOC stock did not differ from RV in SoyEx and MaiEx, while the stock remained stable in CasEx in
1067	2021 (Fig. 3). Because the SOC stock under CT soils have been depleted over the 70 years after natural
1068	forest conversion to cropland (see the history of land use change in Hok et al., 2015) they represent a
1069	potential C sink. Therefore, the gain of SOC stock under CT could be explained by the annual full
1070	retention of crop residues in MaiEx and SoyEx over the experiment period (Table 1) along with the high
1071	elay content of this oxisol.
1072 1073 1074	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.

based management systems were no longer significantly different from the stocks of natural Cerrado

1075 4.73.4 Carbon and N in size fractions and stabilization processes in NT systems

1061

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). In all the experiments, At 0–10 em, NT systems significantly increased C in
both -POM and -by 115%, 118%, in MaiEx and SoyEx, respectively, and by 37% in CasEx although this

1081 was not significant. NT systems also significantly enhanced C-MAOM fractions by 33%, 21%, at in the

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1082	topsoil 0-10 cm-layer (Fig. 4). depth in MaiEx and SoyEx and even deeper to the soil profile > 20 cm in	
1083	CasEx (Fig. 4). These increases could be attributed to the continuous supply of large amounts and diverse	
1084	biomass-C inputs to the soil surface, through the diversity of the root systems along with the low level of	
1085	soil disturbance under NT systems (Sá et al., 2014; Briedis et al., 2018).	Formatted: Font color: Auto
1086	During the decomposition process, microbial communities use the rapidly decomposable materials as	
1087	energy sources, while the recalcitrant and other labile compounds materials act as the glue to bind soil	
1088	mineral particles together (Witzgall et al., 2021). This process is a pathway for the formation of soil micro	
1089	aggregates (Bot and Benites, 2005). The continuous supply of biomass C inputs to the soil associated with	
1090	microbial decomposition without soil mechanical disturbance creates a favourable environment for the	
1091	emergence of soil macroaggregates (Crews and Rumsey, 2017). Organic carbon inside soil aggregates is	
1092	physically protected from microbial oxidation as well as strongly associated with the organo-minerals,	
1093	leading to SOC stabilization over time (Powlson et al., 1987; Lützow et al., 2006). In the same	
1094	experiments as in our study but after 3 years of CA adoption, Hok et al., (2021) reported that soil	
1095	aggregation was one of the main stabilization mechanisms, providing physical protection to the newly	
1096	derived C into the soil microaggregates protected by macroaggregates. From our knowledge of the	
1097	literature, the high SOC accumulation rate recorded under cassava-based CA cropping systems is	
1098	relatively unique and, in addition to the residues of cover crops and maize under the bi-annual crop	
1099	rotation system, the nature of the cassava residues that was retained into the field with high cellulose and	
1100	lignin contents may explain this result (Veiga et al., 2016).	
1101	From an incubation of labelled litter, Witzgall et al., (2021) found that the occlusion of organic matter	Formatted: Font color: Auto
1102	into aggregates and the formation of organo-mineral associations occurs concurrently on fresh litter	

1103	surfaces regardless of soil structure. In addition, the increase in C-MAOM is attributed to C transfer from
1104	POM and other labile C pools. Over time, these compounds are transferred to more stable pools, creating
1105	associations with mineral colloids, with MAOM being more stabilized (Briedis et al., 2018). Rosolem et
1106	al. (2016) conducted 3-year successive experiments to assess the above- and belowground effects of a
1107	wide range of tropical grasses and legume cover crops, which were the same species that were used under
1108	the NT systems in our experiments, in combination with no-till soybean-based cropping systems in
1109	Brazilian tropical elayed clayey Rhodic Ferralsol on total organic C and N stocks and in POM fraction.
1110	They reported that the presence of C4 deep-root grass cover crops during the fallow period significantly
1111	increased total organic C and POM. Furthermore, legume cover crops contributed to maintaining the C/N
1112	ratio in the topsoil layers, which could keep increasing C over time. Beside the aboveground biomass,
1113	root systems of cover crops are also an important C inputs for SOC accumulation due to their capacity to
1114	grow deeper in the soil profile, during the dry season for some, exploring large volume of soil (Rosolem
1115	et al., 2016; Sokol et al., 2019), releasing large quantities of roots exudates, and recycling nutrients
1116	(Rosolem et al., 2005). The increase of in-C stock in -POM and C-MAOM shows that NT systems with
1117	the use of cover crops is a key strategy to promote both SOC storage and long-term SOC stabilization.
1118	In contrast to C-POM and C-MAOM, although the significant increaseour results showed of N-POM
1119	stock in the top 0-10 cm was observed under all the that NT cropping systems only increased the amount
1120	of Nin MaiEx and SoyEx, the depletion of N-MAOM stock was observed below 5 cm in CasEx and below
1121	40 cm in MaiEx and SoyEx-POM. This raises questions about the N dynamics and N supplies through
1122	the use of mineral fertilizers, as well as N fixation through the use of legume crops in the NT cropping
1123	systems. Therefore, there is a need to conduct further research on N use efficiency, N cycles, and nutrient 86

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1124	availability and their stoichiometry relationship by considering deeper layers (> 100 cm) to understand
1125	the mechanism driving N loss under NT systems in these long-term experimentsat the uppermost soil
1126	surface (0-10 cm) in MaiEx and SoyEx over the past 10 years. However, significant decreases in N-POM
1127	and N-MAOM were observed below 40 cm and 5 cm, respectively (Fig. 5).

1128 5 Conclusion

1	129	The present study showed that, over 10 years, variable effects of -were observed among the three-NT
1	130	systems on SOC and TN stocks and pools fractions varied across and in-the three NT systems and the
1	131	experiments. All the NT cropping systems significantly increased SOC stock in the surface layers in
1	132	SoyEx and distributed it toin deeper soil layers under MaiEx and CasEx. Considering layer by layer, the
1	133	significant effect of NT systems on SOC was observed in the topsoil 0-10, 0-20 and 0-40 cm in SoyEx,
1	134	MaiEx, and CasEx, respectively. When considering the whole profile (0-100 cm), the annual SOC
1	135	accumulation we layers, all the NT systems significantly increased SOC stock across the soil profile under
1	136	MaiEx and CasEx. For SoyEx, the cumulative SOC stock is restricted to 0 20 cm under NT
1	137	monocropping and to 0 80 cm under NT crop rotation systems. In the whole profile (0 100 cm), the
1	138	annual SOC cumulative-rates in NT systems ranged from 0.86–1.47, 0.65–1.00, and 0.70–1.07 Mg C ha
1	139	¹ yr ⁻¹ in MaiEx_ , SoyEx, and CasEx, respectively , and from 0.65–1.00 Mg C ha⁺ yr⁺ in. SoyEx despite
1	140	insignificance. Similarly, under all NT systems, the impact of CA cropping systems on thean-increases
1	141	in SOC-POM and C-MAOM stocks were observed in the topsoil layers in MaiEx and SoyEx-under NT
1	142	cropping systems whereas a significant effect on and in C-MAOM stock in soils under CA systems was
1	143	found from the surface to in 0-40 cm in CasEx. In contrast to SOC stock, over the past 10 years, However,
		87

1144	under all the NT systems, N-POM stock NT only increased in the surface 0–10 cm layer, . These systems
1145	did not increase N stock in POM and MAOM either even in the surface layer, but TN-MAOM stock
1146	decreased below 5 cm in CasEx and below 40 cm in MaiEx and SoyEx.it_The main impact of NT systems
1147	on C-POM and C-MAOM was observed in the top 0-10 cm in MaiEx and SoyEx, whereas significant
1148	effect on C-MAOM in soils under NT systems was found from 0-40 cm in CasEx.
1149	In contrast to SOC, N concentration and stock in NT soils only increased in the surface layer (0-5 cm).
1150	Although an increase of N-POM under NT systems was found in the top soils, a decrease was observed
1151	in the subsurface layers. Surprisingly, intensive NT systems caused the depletion of N-MAOM with
1152	significant losses observed below 5, 20, and 40 cm in CasEx, MaiEx and SoyEx, respectively. This
1153	resulted in significant N stock depletion below 40 cm and in the whole profile, particularly, under soybean
1154	and cassava NT monocropping systems.
1155	Overall, our findings reveal that diachronic sampling is crucial for proper measurements of the impacts
1156	of NT systems on SOC dynamics with time. Long-term aAdoption ofing NT cropping systems
1157	accompanied by diversified crop and cover crop species and high biomass C inputs with an absence of
1158	heavy soil disturbance in the long-term significantly increased SOC stock and pools-fractions in the
1159	tropical red Oxisol of Cambodia. The study highlights the potential of NT cropping systems for SOC
1160	accumulation and stabilization over time, even for cassava, which is known to have severe environmental
1161	impacts and induce soil degradation, but raises questions about soil N dynamics. Further research on the
1162	N dynamics N use efficiency, N cycles, and nutrient availability and their stoichiometry relationship by

1163 considering deeper layers (> 100 cm) is needed to understand the mechanism driving N loss in NT systems

1164 for making informed decisions regarding sustainable soil fertility management and crop production

1165 systems.

166 6 Code and data availability

1167 All data are freely available on the CIRAD data repository https://doi.org/10.18167/DVN1/NNBBAQ 1168 (Leng et al., 2024).

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1169 7 Author contributions

VL co-established and managed the experiments, carried out the fieldworks, managed all sample 1170 collection campaigns and laboratory works, and wrote the first manuscript draft. FT co-managed the 1171 1172 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 1173 operations, analytical procedure, data computation, and reviewed the manuscript. VS, PL, LH, JS and CB 1174 gave advices for the analytical procedures, data calculation and manuscript improvement. PM 1175 1176 significantly contributed to the implementation of the field operations, sample collection and lab works. 1177 TF performed the statistical analysis. SB raised funds, designed, established and managed the experiments 1178 during the first years and contributed to the manuscript improvement.

1179 8 Competing interests

180 One co-author is a member of the editorial board of SOIL. The peer review process was guided by an

1 81 independent editor, and the authors declare that they have no competing interests to declare.

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1192 10 References

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