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Stable carbon and nitrogen isotopic composition of leaves, litter, and soils of various ecosystems along an elevational and land-use gradient at Mount Kilimanjaro, Tanzania

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Fertilizer and pesticide isotopic composition

Fertilizers

A general indication of fertilizer used in the region is provided here.

Giller et al. (1998) reported an estimate of ca. 40 kg N ha^{-1} inorganic fertilizer use in the Kilimanjaro region. A more recent report by Senkoro et al. (2017) indicate a generic fertilizer use of 17 kg ha^{-1} y^{-1} on a country basis, with about 12% of the national fertilizer share being used in the Kilimanjaro and Arusha regions. Urea (48% N) and diammonium phosphate (18% N) accounted for about half the total volume of fertilizer used in 2010. The nitrogen isotopic values of both fertilizers is ~0 ‰ (Bateman and Kelly, 2007), and as such does not pose a significant additional bias on the interpretation of soil $\delta^{15}N$ values. However, the addition of manure ($\delta^{15}N$ ~8 ‰) in Hom systems, albeit used in low quantities (Gütlein et al., 2018), may have well contributed to the high $\delta^{15}N$ values observed in this ecosystem (Fig. 4).

Bateman, A. S., and Kelly, S. D. (2007). Fertilizer nitrogen isotope signatures. *Isotopes in environmental and health studies*, *43*(3), 237-247.

Giller et al. (1998). Environmental constraints to nodulation and nitrogen fixation of Phaseolus vulgaris L in Tanzania II. Response to N and P fertilizers and inoculation with Rhizobium. *African Crop Science Journal*, *6*(2), 171-178.

Gütlein et al (2018). Impacts of climate and land use on N_2O and CH_4 fluxes from tropical ecosystems in the Mt. Kilimanjaro region, Tanzania. Glob. Change Biol. 24, 1239–1255.

Senkoro et al (2017). Optimizing fertilizer use within the context of integrated soil fertility management in Tanzania. *Fertilizer use optimization in Sub-Saharan Africa. CAB International, Nairobi, Kenya*, 176-192.

Pesticides

The isotopic values of the two most commonly used pesticides are shown below in Table S1. The actual product values may strongly depend on the manufacturer, which as in the case of $\delta^{13}C$ can be quite different for glyphosate.

Table S1

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Glyphosate	-24.0 ; -34.0 [1]	-3.6 [2]
Atrazine	-28.9 ; -27.9 [3]	-0.2 ; -1.5 [3]

[1] Kujawinski, D. M., Wolbert, J. B., Zhang, L., Jochmann, M. A., Widory, D., Baran, N., & Schmidt, T. C. (2013). Carbon isotope ratio measurements of glyphosate and AMPA by liquid chromatography coupled to isotope ratio mass spectrometry. *Analytical and bioanalytical chemistry*, *405*(9), 2869-2878.

[2] Tavares, C. R. D. O., Bendassolli, J. A., Ribeiro, D. N., & Rossete, A. L. R. M. (2010). ^{15}N-labeled glyphosate synthesis and its practical effectiveness. *Scientia Agricola*, *67*(1), 96-101

[3] Meyer, A. H., Penning, H., Lowag, H., & Elsner, M. (2008). Precise and accurate compound specific carbon and nitrogen isotope analysis of atrazine: critical role of combustion oven conditions. *Environmental science & technology*, *42*(21), 7757-7763.

Table S2 Pearson's correlations coefficients (r) between soil, litter, leaf, and climatic parameters. Correlation analysis was conducted with all five replicates of each of the twelve ecosystems (n = 60)

	Variable	Soil					Litter					Leaf				
		$\delta^{15}N$	N content	$\delta^{13}C$	C content	C/N ratio	$\delta^{15}N$	N content	$\delta^{13}C$	C content	C/N ratio	$\delta^{15}N$	N content	$\delta^{13}C$	C content	C/N ratio
Soil	$\delta^{15}N$		-0.70***	0.52***	-0.76***	-0.54***	0.82***	-0.13	0.44***	-0.72***	-0.06	0.75***	0.21	0.47***	-0.38**	-0.27*
	N content			-0.63***	0.96***	0.38**	-0.44***	0.49***	-0.56***	0.72***	-0.26*	-0.38**	0.21	-0.61***	0.34**	-0.15
	$\delta^{13}C$				-0.61***	0.01	0.18	-0.60***	0.79***	-0.43***	0.51***	0.15	-0.31*	0.76***	-0.49***	0.28*
	C content					0.56***	-0.53***	0.38**	-0.54***	0.76***	-0.17	-0.45***	0.07	-0.59***	0.42***	-0.04
	C/N ratio						-0.59***	-0.19	-0.15	0.51***	0.303*	-0.54***	-0.40**	-0.14	0.39**	0.40**
Litter	$\delta^{15}N$							0.26*	0.13	-0.68***	-0.48***	0.92***	0.53***	0.20	-0.25	-0.57***
	N content								-0.66***	0.26*	-0.87***	0.26*	0.73***	-0.61***	0.21	-0.64***
	$\delta^{13}C$									-0.42***	0.54***	0.14	-0.36**	0.88***	-0.54***	0.22
	C content										0.11	-0.57***	-0.05	-0.49***	0.39**	0.08
	C/N ratio											-0.42***	-0.69***	0.47***	-0.12	0.63***
Leaf	$\delta^{15}N$												0.53***	0.17	-0.17	-0.61***
	N content													-0.44***	-0.13	-0.92***
	$\delta^{13}C$														-0.44***	0.30*
	C content															0.19
	C/N ratio															
Soil	pH	0.51***	-0.76***	0.65***	-0.78***	-0.28*	0.26*	-0.51***	0.44***	-0.55***	0.34**	0.20	-0.24	0.45***	-0.40**	0.26*
	clay content	0.14	0.33**	-0.23	0.27*	-0.10	0.32*	0.37**	-0.12	0.02	-0.34**	0.31*	0.44***	-0.16	-0.06	-0.46***
	silt content	0.01	0.27*	-0.04	0.30*	0.20	0.08	0.22	0.02	0.14	-0.23	0.09	0.15	-0.01	0.05	-0.24
	sand content	-0.12	-0.43***	0.22	-0.39**	-0.04	-0.31*	-0.43***	0.09	-0.10	0.41**	-0.31*	-0.45***	0.14	0.02	0.52***
MAP		-0.60***	0.81***	-0.72***	0.76***	0.19	-0.32*	0.58***	-0.65***	0.50***	-0.44***	-0.27*	0.33**	-0.60***	0.34**	-0.26*
MAT		0.73***	-0.54***	0.66***	-0.60***	-0.33**	0.67***	-0.16	0.55***	-0.62***	0.05	0.61***	0.25	0.55***	-0.48***	-0.33*

Levels of significance: * P < 0.05, ** P < 0.01, *** P < 0.001

Table S3 Correlation coefficients (r) and P values of selected variables included in the principal component analysis used to identify the main factors driving soil $\delta^{15}N$. Only variables showing r > 0.5 were considered

Principal component	**Variable**	**r**	**P value**
PC 1	Soil C content	0.93	<0.001
	Soil N content	0.93	<0.001
	Soil C/N ratio	0.61	<0.001
	Soil pH	-0.87	<0.001
	Soil $\delta^{13}C$	-0.76	<0.001
	MAP	0.87	<0.001
	MAT	-0.63	<0.001
PC 2	Soil clay content	-0.84	<0.001
	Soil sand content	0.82	<0.001
	MAT	-0.65	<0.001

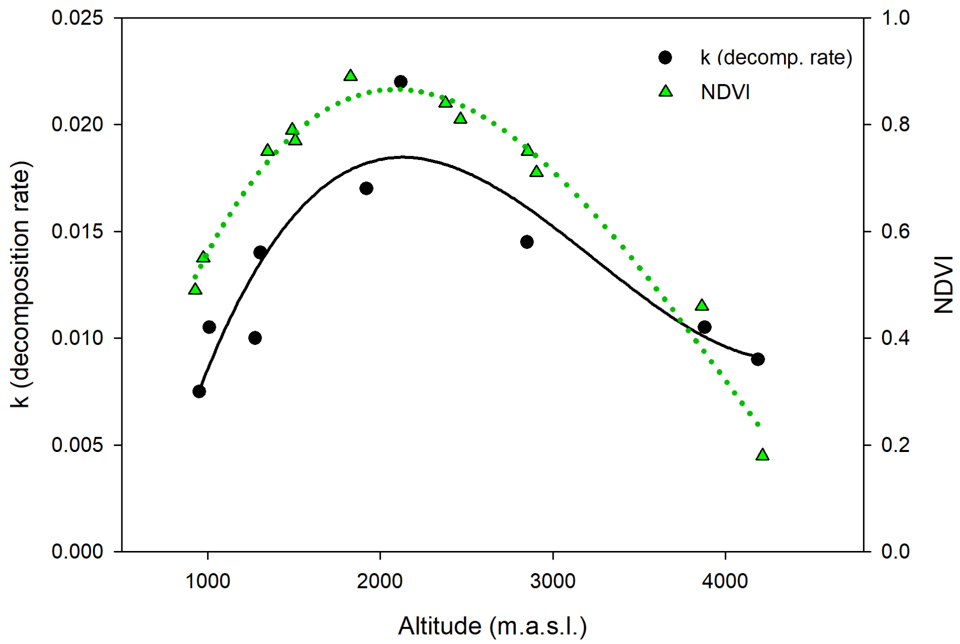

Fig. S1 Annual means of Tea Bag Index decomposition rate constant (k) reported by Becker and Kuzyakov (2018), and Normalized Difference Vegetation Index (NDVI) calculated by Röder et al. (2017) as a proxy for primary productivity (Kerr and Ostrovsky, 2003) for the same ecosystems studied in the Kilimanjaro land-use and elevational gradient. Solid and dotted line corresponds to k and NDVI 3rd degree polynomial regressions; r^2 0.82 and 0.78 respectively.

References:

Becker, J. N., and Kuzyakov, Y. (2018). Teatime on Mount Kilimanjaro: Assessing climate and land-use effects on litter decomposition and stabilization using the Tea Bag Index. *Land Degradation & Development*, *29*(8), 2321-2329.

Kerr, J. T., and Ostrovsky, M. (2003). From space to species: ecological applications for remote sensing. *Trends in ecology & evolution*, *18*(6), 299-305.

Röder, J., Detsch, F., Otte, I., Appelhans, T., Nauss, T., Peters, M. K., & Brandl, R. (2017). Heterogeneous patterns of abundance of epigeic arthropod taxa along a major elevation gradient. *Biotropica*, *49*(2), 217-228.

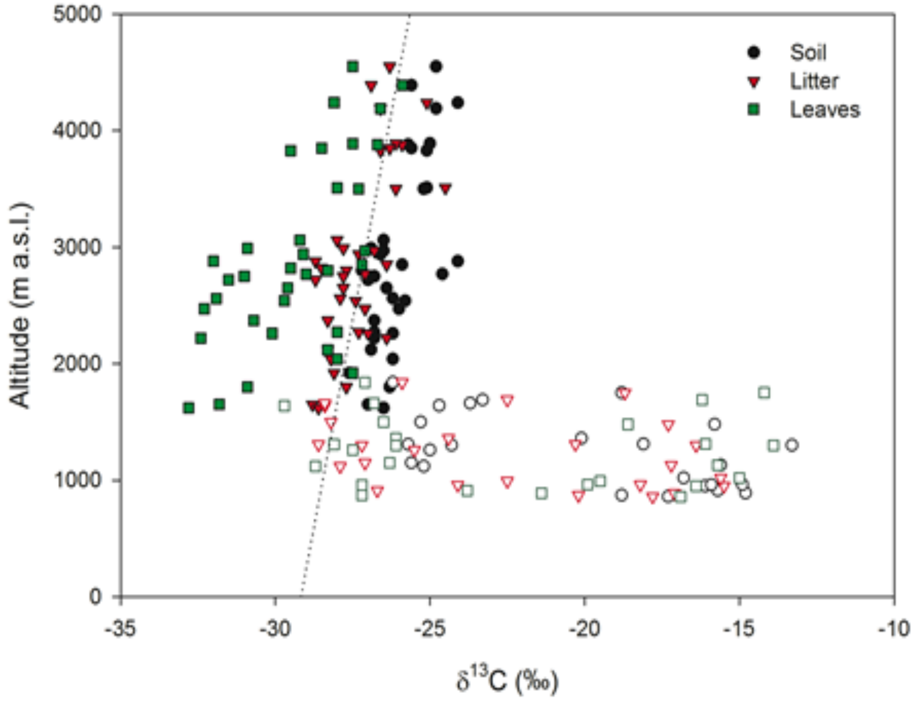

Fig. S2 Variation in $\delta^{13}C$ values along the Kilimanjaro land-use and elevational gradient for leaves, litter, and soil. Solid symbols denote semi-natural ecosystems, while open symbols correspond to managed ecosystems. The dotted line represents the theoretical global relationship between altitude and $\delta^{13}C$ of plant leaves (C_3 vegetation only) developed by Körner et al. (1988) and is shown here for reference. The ecosystem acronyms used are as per Table 1. Mai, Cof, and Hom are managed cropping sites, Gra and Sav are extensively managed grasslands and savannas, while the rest represent semi-natural ecosystems. *Reference:* Körner, C., Farquhar, G.D., Roksandic, Z., 1988. A global survey of carbon isotope discrimination in plants from high altitude. Oecologia 74, 623–632. https://doi.org/10.1007/BF00380063.

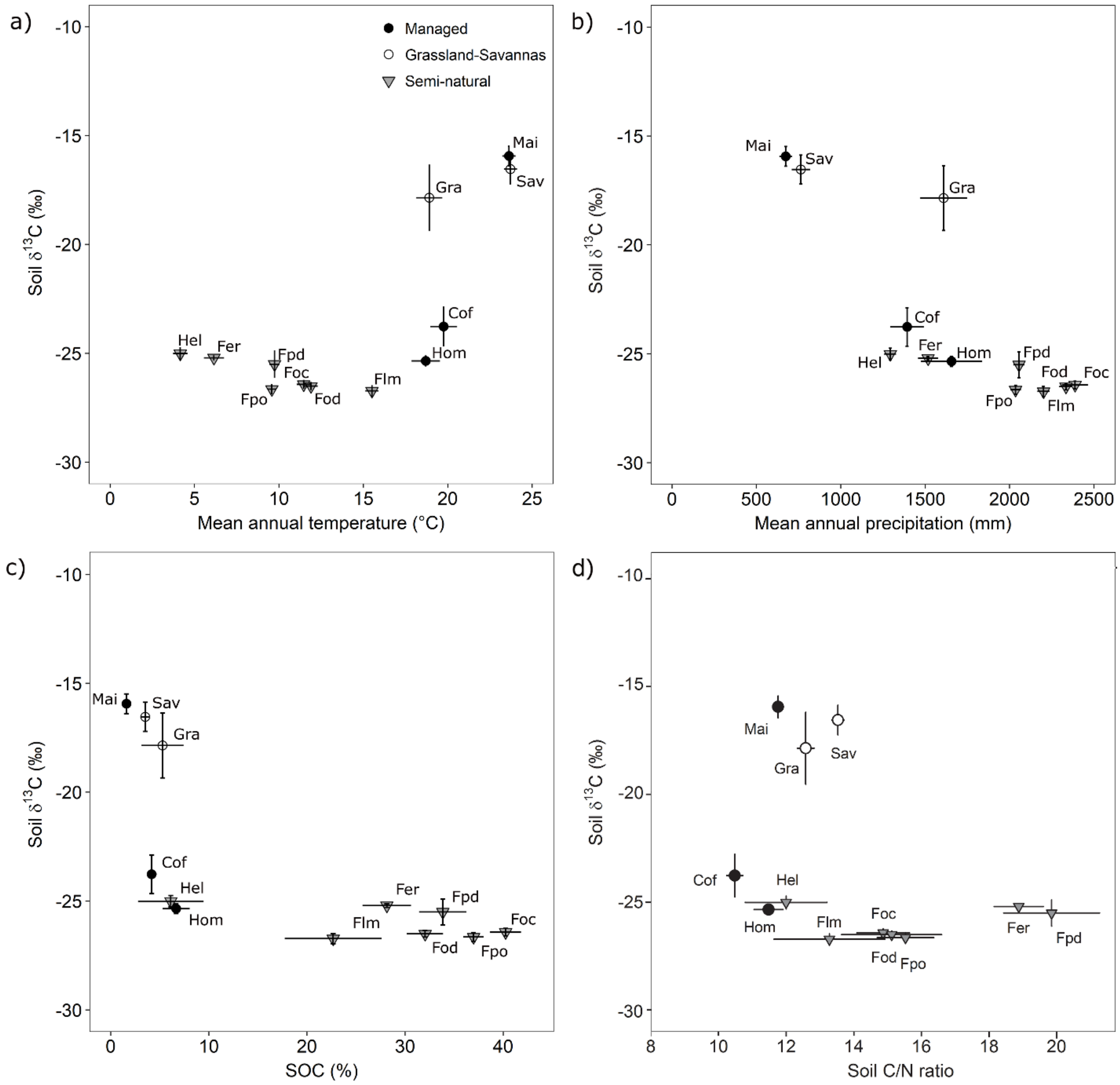

Fig. S3 Relationship between soil $\delta^{13}C$ values and mean annual temperature (a), mean annual precipitation (b), soil organic carbon (c), and soil C/N ratios (d) for all ecosystems. Each data point represents the average of five sites, and bars denote standard error of the means. Symbols are as per all previous figures. The ecosystem acronyms used are as per Table 1.

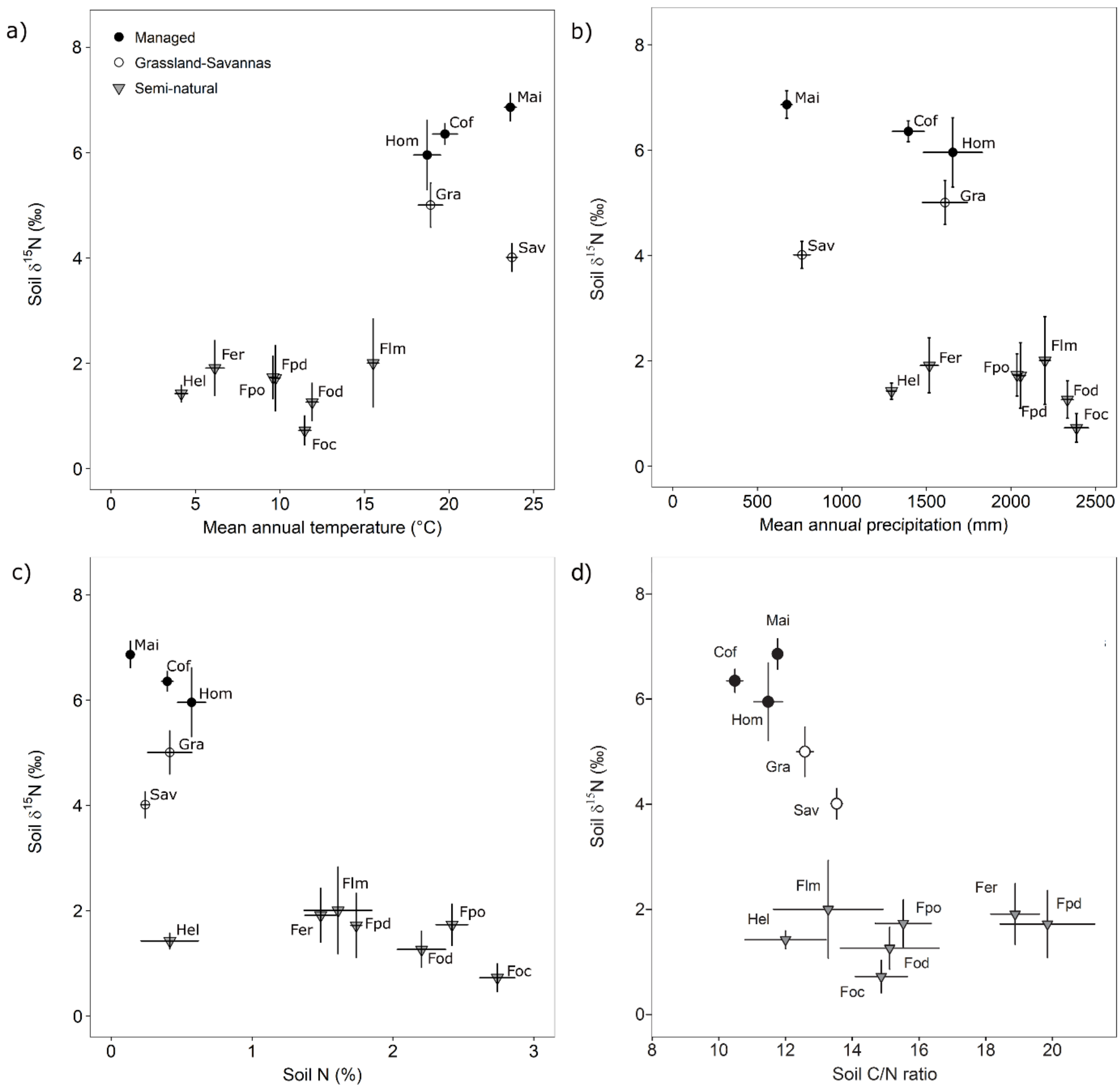

Fig. S4 Relationship between soil $\delta^{15}N$ values and mean annual temperature (a), mean annual precipitation (b), soil nitrogen (c), and soil C/N ratios (d) for all ecosystems. Each data point represents the average of five sites, and bars denote standard error of the means. Symbols are as per all previous figures. The ecosystem acronyms used are as per Table 1.