Enzyme kinetics inform about mechanistic changes in tea litter decomposition across gradients in land-use intensity in Central German grasslands

Journal Article

Author(s):
Meyer, Ulf-Niklas; Tischer, Alexander; Freitag, Martin; Klaus, Valentin; Kleinebecker, Till; Oelmann, Yvonne; Kandeler, Ellen; Hölzel, Norbert; Hamer, Ute

Publication date:
2022-08-25

Permanent link:
https://doi.org/10.3929/ethz-b-000546310

Originally published in:
Science of The Total Environment 836, https://doi.org/10.1016/j.scitotenv.2022.155748

Appendix

Enzyme kinetics inform about mechanistic changes in tea litter decomposition across gradients in land-use intensity in Central German grasslands

Ulf-Niklas Meyer[a], Alexander Tischer[b], Martin Freitag[a], Valentin H. Klaus[c], Till Kleinebecker[d], Yvonne Oelmann[e], Ellen Kandeler[f], Norbert Hölzel[a], Ute Hamer[a*]

[a]Institute of Landscape Ecology, University of Münster, Heisenbergstraße 2, 48149 Münster, Germany

[b]Department of Soil Science, Friedrich-Schiller University Jena, Löbdergraben 32, 07743 Jena, Germany

[c]Insitute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland

[d]Institute for Landscape Ecology and Resource Management, Giessen University, Heinrich-Buff-Ring 26-32, 35392 Gießen, Germany

[e] Geoecology, Department of Geosciences, University of Tübingen, Rümelinstr. 19-23, 72070 Tübingen, Germany

[f] Institute of Soil Science and Land Evaluation, Department of Soil Biology, University of Hohenheim, Emil Wolff Str. 27, 70599 Stuttgart, Germany

*Corresponding author: ute.hamer@uni-muenster.de

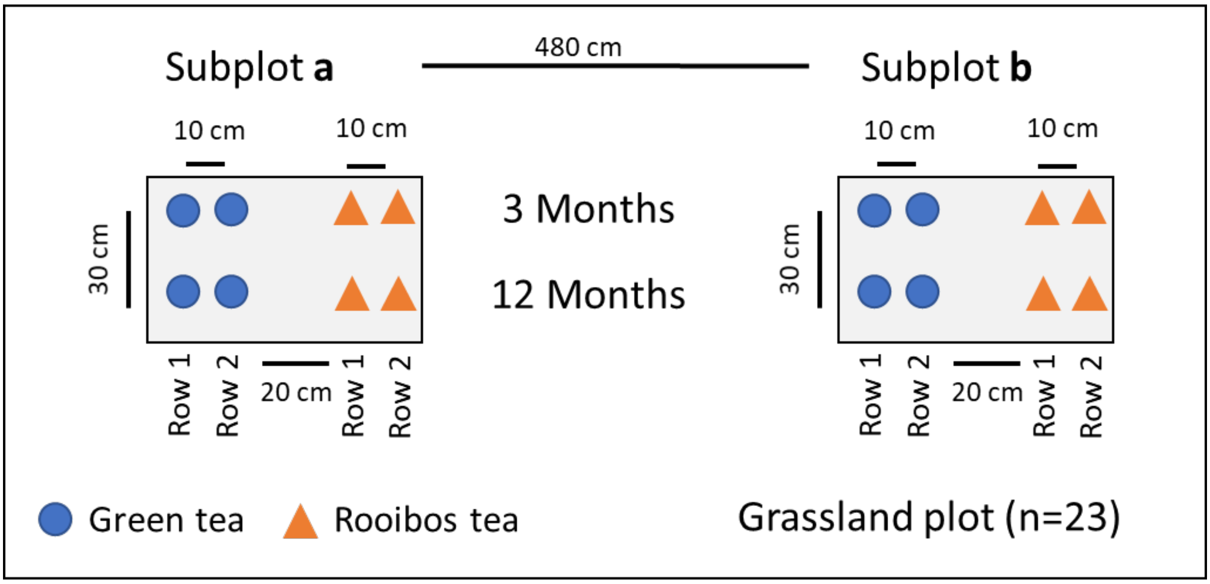


Figure A.1: **Experimental design** established on 23 grasslands with different land-use intensity. In total, eight tea bags containing either green tea or rooibos tea were buried in topsoil (5 cm soil depth) of subplot a and b. The distance between the two tea types was at least 20 cm. Within a tea type, the distance between the **a** and **b** samples was 10 cm. Between the tea bags of different months (3 months, 12 months) the distance was 30 cm. After three months, as well as after twelve months, four green tea bags and four rooibos tea bags were excavated. For analysis of enzyme kinetics, tea litter buried in row 1 and 2 was pooled per months and per subplot.

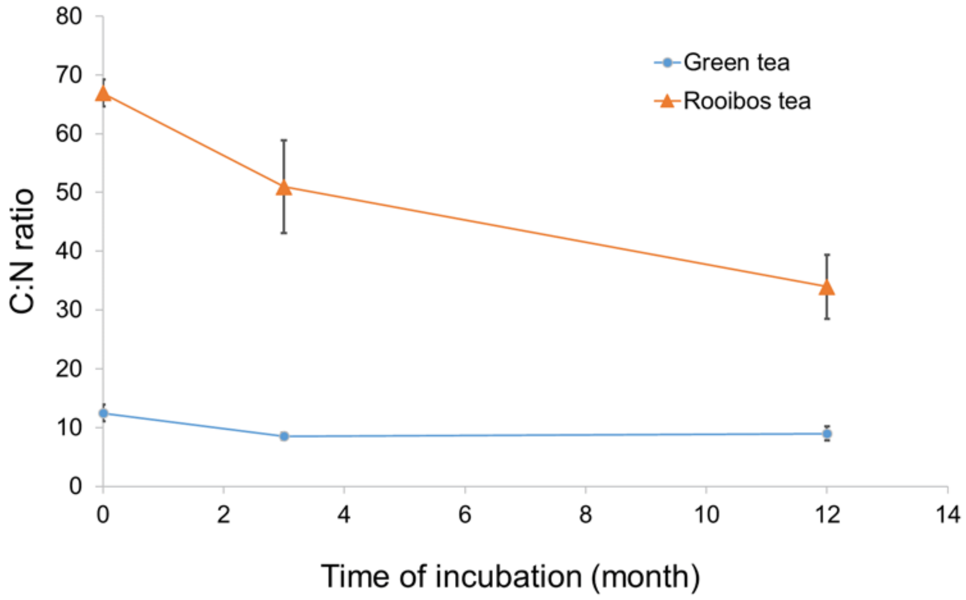


Figure A.2: **C/N ratio change over time.** Ratio of total Carbon to total Nitrogen in tea litter before incubation and after three and 12 months of exposure in five selected grassland soils with different land-use intensity (mean values, bars represent standard deviation).

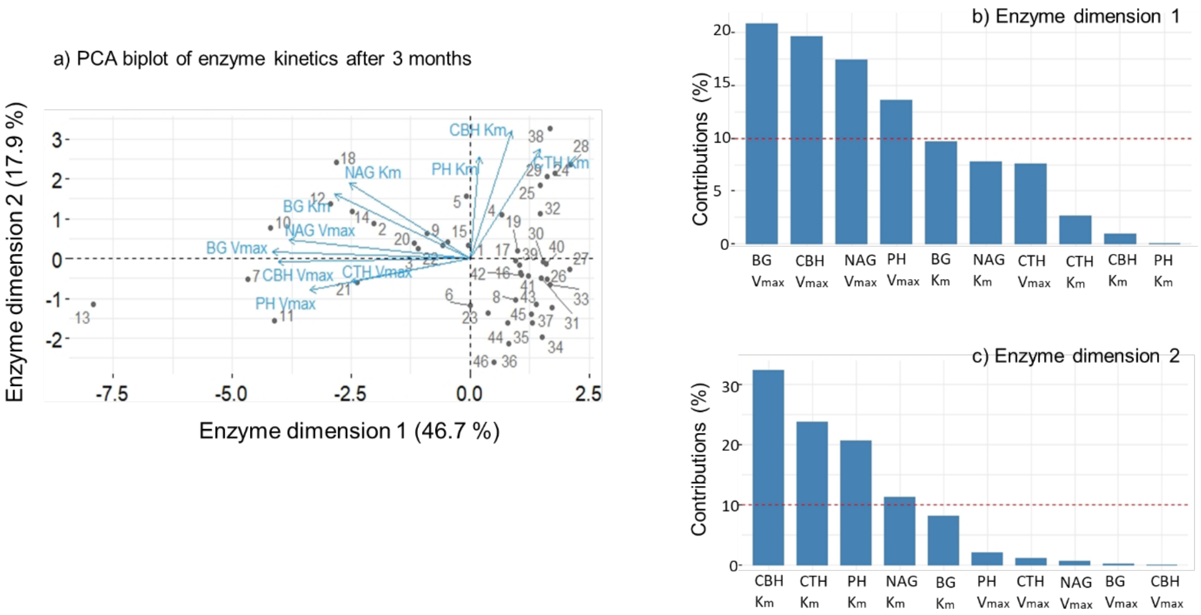


Figure A.3: **Enzyme kinetics dimension reduction.** Principal component analysis (PCA) (a) of the enzyme kinetics (V_{max}, K_m) in tea bags after three months of decomposition used for dimension reduction. Numbers indicate the different grassland plots with green tea (1-23) or rooibos tea (24-46). The first dimension (enzyme dim.1) (b) represents the V_{max} values of BG (β-glucosidase), CBH (cellobiohydrolase), NAG (N-acetyl-β-glucosidase), PH (phosphatase). The second dimension (enzyme dim.2) (c) represents K_m values of CBH, CTH (Cellotriohydrolase), PH, NAG and BG. Both dimensions were used in the linear mixed-effects model to model litter mass loss after three months (Table 1).

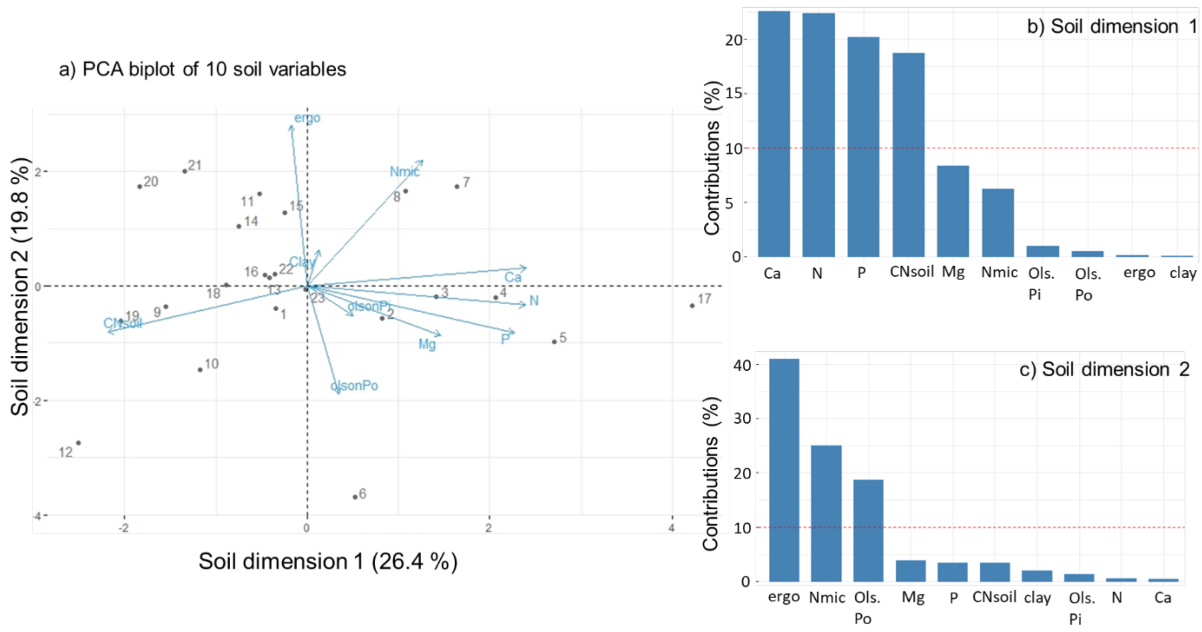


Figure A.4: **Soil parameter dimension reduction.** Principal component analysis (PCA) (a) of the soil variables (resin-NO_3-N + NH_4-N (N), resin-Ca (Ca), resin-PO_4-P (P), resin-Mg (Mg), Olsen-Pi, Olsen-Po, soils C:N ratio, MBN (N_{mic}), ergosterol (ergo), and clay content) used for dimension reduction. Numbers 1 to 23 indicate the different grassland plots. The first dimension (soil dim.1) (b) represents the soil nutrients and includes resin-Ca, resin-N, resin-PO_4-P, as well as the soil organic carbon to nitrogen ratio (C:N ratio). The second dimension (soil dim.2) (c) represents mainly soil microbial properties and includes ergosterol, a fungal marker, and microbial nitrogen (MBN). Both dimensions were used in the linear mixed-effects models (Table 1).

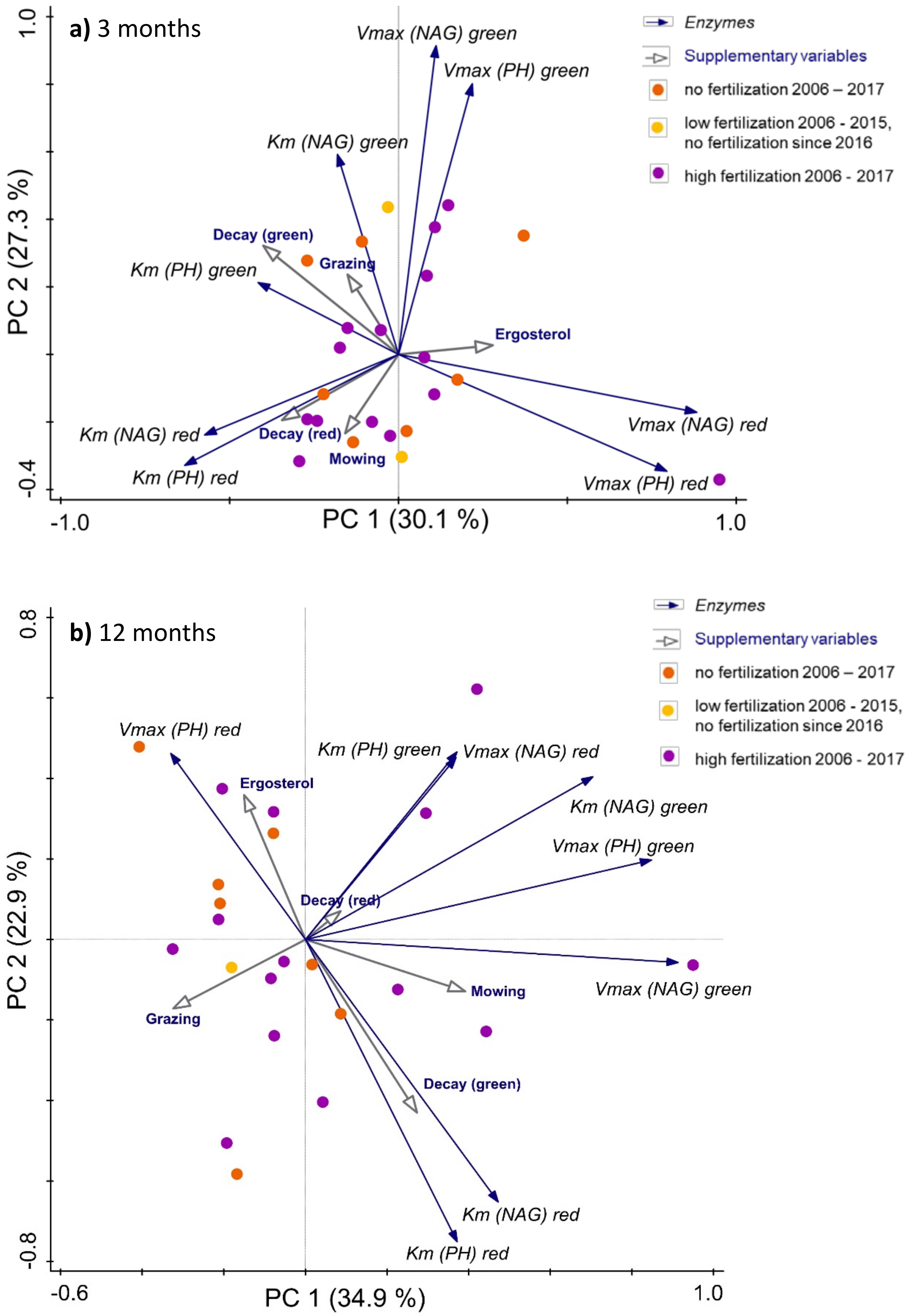
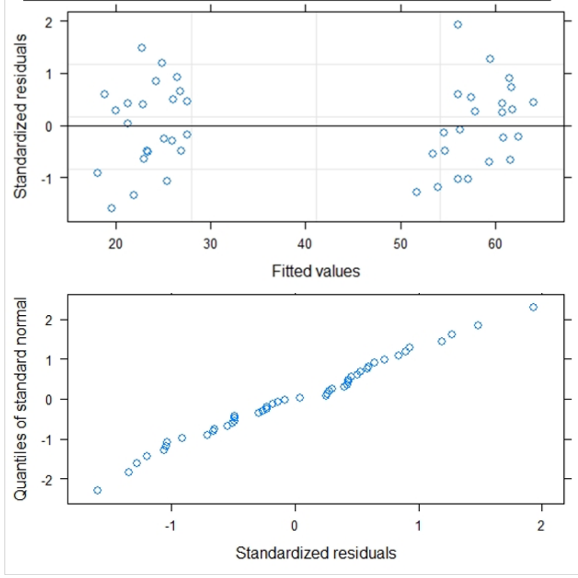


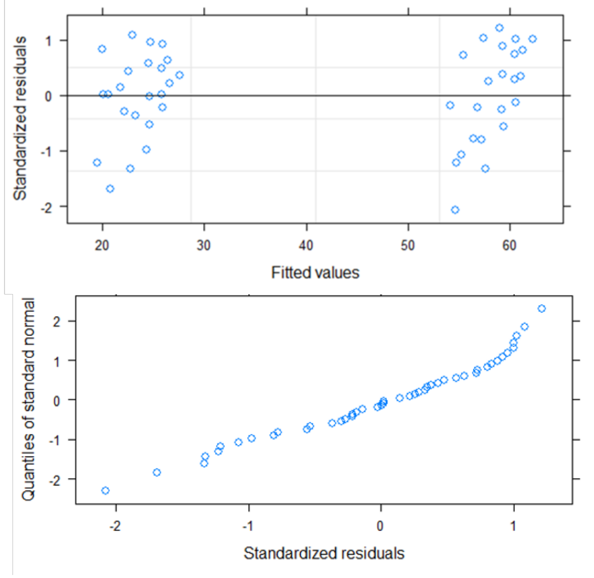
Figure A.5: **Principal component analysis (PCA)** of the V_{max} and K_m values of the N- and P-cycle enzymes (NAG, PH) in litter of green or rooibos tea after a) three months decomposition and b) twelve months decomposition in the topsoil of 23 grassland sites. Grazing and mowing intensity during the last 10 to 11 years, respectively, as well as mass loss of green tea (decay green) and rooibos tea (decay red) are shown as supplementary variables. Orange circles are plots with no fertilization, yellow circles are plots with low fertilization and purple circles are plots with high fertilization a)

3 Months „best“	Estimate	LL	UL
(Intercept)	53.855	50.632	57.078
tea type	**-33.730**	**-35.873**	**-31.588**
mowing long	**3.154**	**1.265**	**5.042**
grazing long	1.307	-0.500	3.113
soil dim. 2	-1.015	-1.967	0.064
enzyme dim. 1	**0.640**	**0.183**	**1.097**
enzyme dim. 2	-0.210	-0.804	0.384
tea type x mowing long	-0.812	-2.385	0.761
Observations	46		
Akaike Inf. Crit.	250.3		
R²m	0.9670		
R²c	0.9860		



b)

3 Months	Estimate	LL	UL
(Intercept)	58.251	56.552	59.949
tea type	**-34.604**	**-36.276**	**-32.932**
Observations	46		
Akaike Inf. Crit.	258.3		
R²m	0.9520		
R²c	0.9767		



c)

3 Months	Estimate	LL	UL
(Intercept)	54.809	51.912	57.707
tea type	**-34.109**	**-36.765**	**-31.453**
mowing long	**2.476**	**0.733**	**4.219**
grazing long	1.012	-0.548	2.574
soil dim. 2	**-0.904**	**-1.731**	**-0.078**
tea type x mowing long	-0.459	-.2381	1.461
Observations	46		
Akaike Inf. Crit.	255.1		
R²m	0.9686		
R²c	0.9769		

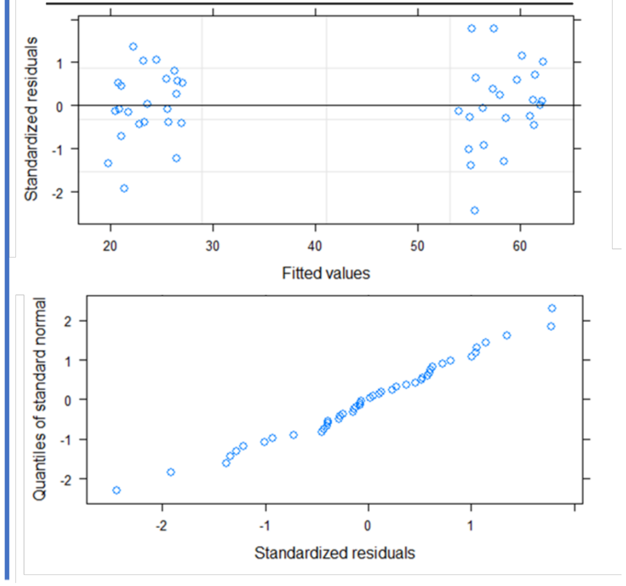


Figure A.6: **Linear mixed-effects models** (nlme package, (Pinheiro et al. 2020)) to find the best set of predictor variables (land-use intensity, enzyme kinetics, basic soil characteristics) that explain tea litter mass loss [%] after three months (n = 46) on 23 grassland sites. Estimated effect sizes and lower (LL) and upper (UL) CIs are shown as well as statistical quality features of the “best” model presented in Table 1 (a), the first simple model (b) and a complex model without enzyme characteristics (c). R²m accounts for that proportion of variance that is explained by the fixed effects while R²c accounts for the sum of variance explained by fixed and random effects. Thus, the difference between R²c and R²m is a measure of the proportion of variance explained by plot. Enzymatic and soil data that were used in PCA (enzyme dim. 1 and 2; soil dim. 1) were centered and standardized by norm.

a)

12 Months „best“	Estimate	LL	UL
(Intercept)	63.427.	61.001	65.845
tea type	**-29.609**	**-33.201**	**-26.017**
grazing long	**-1.507**	**-3.601**	**0.587**
soil dim. 1	0.320	-0.601	1.241
soil dim. 2	-0.507	-1.485	0.471
CBH Km	-1.545	-3.157	0.067
CTH Vmax	**1.956**	**0.210**	**3.701**
PH Vmax	**1.914**	**0.373**	**3.454**
Observations	34		
Akaike Inf. Crit.	205.6		
R²m	0.9456		
R²c	0.9546		

b)

12 Months	Estimate	LL	UL
(Intercept)	62.222	60.006	64.439
tea type	**-29.79**	**-32.810**	**-26.762**
Observations	44		
Akaike Inf. Crit.	274.4		
R²m	0.9001		
R²c	0.9077		

c)

12 Months	Estimate	LL	UL
(Intercept)	64.235	61.506	66.963
tea type	**-30.001**	**-33.511**	**-26.491**
grazing long	**-2.625**	**-4.943**	**-0.307**
soil dim. 1	-0.263	-1.282	0.755
soil dim. 2	-0.226	-1.334	0.881
Observations	34		
Akaike Inf. Crit.	218.6		
R²m	0.9171		
R²c	0.9171		

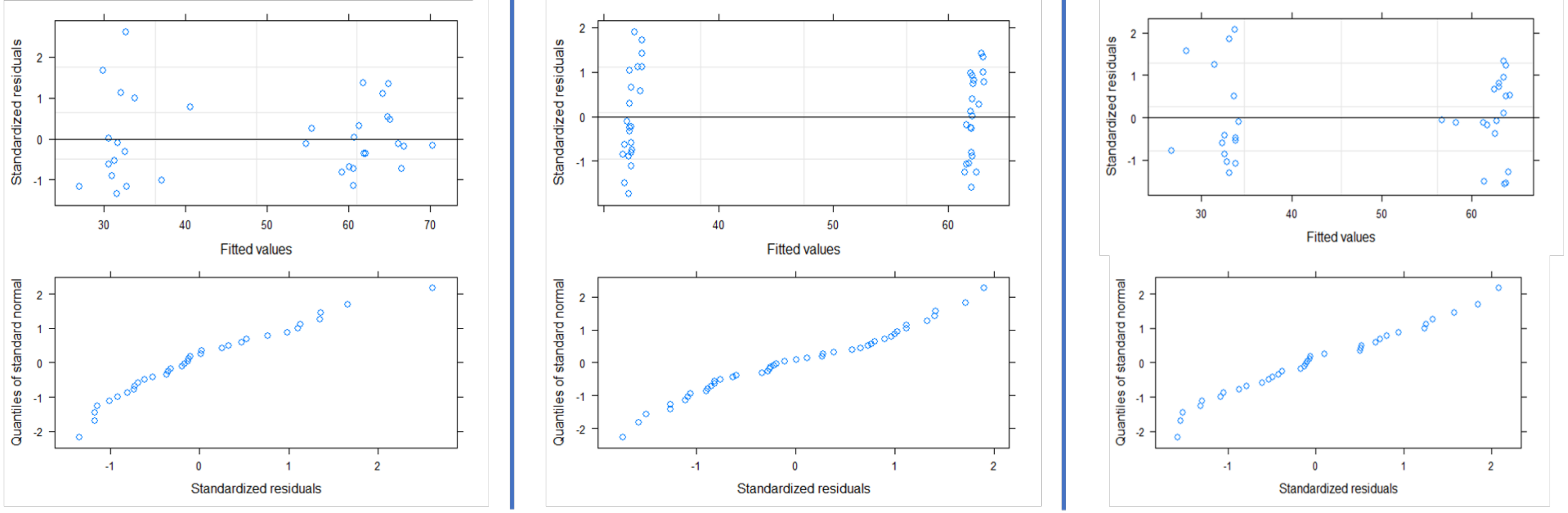


Figure A.7: **Linear mixed-effects models** (nlme package, (Pinheiro et al. 2020)) to find the best set of predictor variables (land-use intensity, enzyme kinetics, basic soil characteristics) that explain tea litter mass loss [%] after twelve months (n = 34) on 23 grassland sites. Estimated effect sizes and lower (LL) and upper (UL) CIs are shown as well as statistical quality features of the “best” model presented in Table 1 (a), the first simple model (b) and a complex model without enzyme characteristics (c). R^2m accounts for that proportion of variance that is explained by the fixed effects while R^2c accounts for the sum of variance explained by fixed and random effects. Thus, the difference between R^2c and R^2m is a measure of the proportion of variance explained by plot. Soil data that were used in PCA (soil dim. 1, soil dim. 2) were centered and standardized by norm.

Table A.1: Basic plot data about coordinates, soil type, land-use type, grazing, mowing, fertilization, and land-use index.

Plot[a]	**Coordinates (WGS 84)**	**Soil type (WRB)**[a]	**Land-use type**[a]	**Grazing 2016**[b,c]	**Mowing [cuts * year^{-1}] 2016**[b,c]	**Fertilization [kg N * ha^{-1}] 2016**[b,c]	**LUI 2016 (2017)**[b,c]
HEG 1	50°58' N, 10°24' E	Cambisol	Meadow	Yes	2	216	3.09 (3.10)
HEG 2	51°0' N, 10°2' E	Vertisol	Meadow	No	2	130	2.25 (2.21)
HEG 3	50°59' N, 10°2' E	Vertisol	Meadow	No	3	130	2.44 (2.32)
HEG 4	51°6' N, 10°26' E	Stagnosol	Mown Pasture	No	2	104	2.00 (1.94)
HEG 5	51°12' N, 10°19' E	Stagnosol	Mown Pasture	No	3	81	2.17 (2.10)
HEG 6	51°12' N, 10°23' E	Stagnosol	Mown Pasture	Yes	2	105	2.35 (2.29)
HEG 7	51°16' N, 10°24' E	Stagnosol	Pasture	Yes	0	0	1.61 (2.00)
HEG 8	51°16' N, 10°25' E	Stagnosol	Pasture	Yes	0	0	1.61 (1.37)
HEG 9	51°13' N, 10°22' E	Stagnosol	Pasture	Yes	1	0	1.27 (1.06)
HEG 10	51°16' N, 10°26' E	Vertisol	Meadow	Yes	1	14	1.86 (1.75)
HEG 11	51°16' N, 10°26' E	Stagnosol	Meadow	Yes	1	14	1.75 (1.54)
HEG 20	51°13' N, 10°22' E	Stagnosol	Pasture	Yes	1	0	1.27 (1.06)
HEG 21	51°11' N, 10°45' E	Stagnosol	Pasture	Yes	1	0	1.42 (1.16)
HEG 26	51°16' N, 10°22' E	Cambisol	Meadow	No	1	0	1.30 (1.53)
HEG 27	51°5' N, 10°35' E	Cambisol	Meadow	Yes	1	60	1.75 (1.76)
HEG 30	51°12' N, 10°21' E	Cambisol	Mown Pasture	Yes	3	71	2.28 (2.15)
HEG 37	51°1' N, 10°30' E	Cambisol	Mown Pasture	Yes	2	84	2.11 (1.81)
HEG 40	50°58' N, 10°26' E	Cambisol	Pasture	Yes	0	0	2.14 (1.75)

HEG 42	51°4' N, 10°27' E	Cambisol	Pasture	Yes	0	0	0.87 (0.57)
HEG 43	51°18' N, 10°26' E	Cambisol	Pasture	Yes	0	0	0.75 (0.56)
HEG 44	51°3' N, 10°28' E	Cambisol	Pasture	Yes	0	0	0.81 (0.72)
HEG 48	51°17' N, 10°22' E	Cambisol	Mown Pasture	Yes	1	0	1.49 (1.54)
HEG 50	51°16' N, 10°25' E	Cambisol	Mown Pasture	Yes	1	0	1.68 (1.57)

[a] Fischer, M., Bossdorf, O., Gockel, S., Hänsel, F., Hemp, A., Hessenmöller, D., Korte, G., Nieschulze, J., Pfeiffer, S., Prati, D., Renner, S., Schöning, I., Schumacher, U., Wells, K., Buscot, F., Kalko, E. K. V., Linsemair, K. E., Schulze, E.-D., Weisser, W. W. (2010): Implementing large-scale and long-term functional biodiversity research. The Biodiversity Exploratories. Basic Appl. Ecol., 11, 6, pp. 473–485, DOI: 10.1016/j.baae.2010.07.009.

[b] Blüthgen, N., Dormann, C.F., Prati, D., Klaus, V.H., Kleinebecker, T., Hölzel, N., Alt, F., Boch, S., Gockel, S., Hemp, A., Müller, J., Nieschulze, J., Renner, S.C., Schöning, I., Schumacher, U., Socher, S.A., Wells, K., Birkhofer, K., Buscot, F., Oelmann, Y., Rothenwöhrer, C., Scherber, C., Tscharntke, T., Weiner, C.N., Fischer, M., Kalko, E.K.V., Linsenmair, K.E., Schulze, E.-D., Weisser, W.W. (2012): A quantitative index of land-use intensity in grasslands. Integrating mowing, grazing and fertilization. *Basic Appl. Ecol.*, 13, 207–220, DOI: 10.1016/j.baae.2012.04.001.

[c] Vogt, J., Klaus, V.H., Both, S., Fürstenau, C., Gockel, S., Gossner, M.M., Heinze, J., Hemp, A., Hölzel, N., Jung, K., Kleinebecker, T., Lauterbach, R., Lorenzen, K., Ostrowski, A., Otto, N., Prati, D., Renner, S., Schumacher, U., Seibold, S., Simons, N., Steitz, I., Teuscher, M., Thiele, J., Weithmann, S., Wells, K., Wiesner, K., Ayasse, M., Blüthgen, N., Fischer, M., Weisser, W.W., 2019. Eleven years' data of grassland management in Germany. Biodiversity data journal 7, e36387, DOI: 10.3897/BDJ.7.e36387.

Table A.2: Enzymes and their substrates used in this study.

Enzyme	Enzyme function	Substrate	EC	Abbreviation
β-glucosidase	C cycle: cellulose decomposition	4-MUF-β-D-glucoside	3.2.1.21	BG
Cellobiohydrolase	C cycle: cellulose decomposition	4-MUF-β-cellobioside	3.2.1.91	CBH
Cellotriohydrolase	C cycle: cellulose decomposition	4-MUF-β-cellotrioside	3.2.1.4	CTH
1,4-β-N-acetylglucosaminidase	C- and N-cycle: chitin and peptidoglycan degradation	4-MUF-2-deoxy-2-acetamido-β-D-glucoside	3.2.1.52	NAG
Phosphatase	P-cycle: hydrolysis of phosphomonoesters	4-MUF-phosphate	3.1.3	PH

Table A.3: Decomposition rate (k) and litter stabilization factor (S) calculated as Tea Bag Index (TBI) according to Keuskamp et al. (2013) using the mass loss of green tea and rooibos tea after 3 months. Individual values of all bags (n=4) per plot (n=23) were shown buried in two rows of two subplots (a, b).

			Mass loss after 3 months		Tea Bag Index	
Plot	**Subplot**	**Row**	**Green tea [%]**	**Rooibos tea [%]**	**S**	**k [d^{-1}]**
HEG 1	a	1	62.2	33.2	0.261	0.019
		2	62.4	27.6	0.259	0.013
	b	1	55.9	26.5	0.336	0.014
		2	60.0	26.3	0.287	0.012
HEG 2	a	1	64.6	29.1	0.233	0.013
		2	66.2	30.0	0.214	0.013
	b	1	62.9	26.7	0.253	0.012
		2	66.1	28.4	0.215	0.012
HEG 3	a	1	57.9	26.5	0.313	0.013
		2	63.3	23.8	0.248	0.009
	b	1	58.6	23.7	0.304	0.011
		2	61.5	24.5	0.269	0.010
HEG 4	a	1	65.6	25.6	0.221	0.010
		2	65.1	26.6	0.226	0.011
	b	1	60.0	24.7	0.287	0.011
		2	62.9	32.0	0.253	0.017
HEG 5	a	1	61.0	26.5	0.276	0.012
		2	61.5	26.8	0.270	0.012
	b	1	66.1	24.8	0.215	0.009
		2	61.2	25.4	0.274	0.011
HEG 6	a	1	62.6	23.2	0.256	0.009
		2	59.7	20.4	0.291	0.008
	b	1	61.6	19.7	0.269	0.007
		2	62.8	29.6	0.255	0.014
HEG 7	a	1	57.0	15.7	0.323	0.006
		2	57.9	13.6	0.312	0.005
	b	1	57.5	18.0	0.317	0.007
		2	57.1	17.6	0.321	0.007
HEG 8	a	1	50.0	25.5	0.406	0.017
		2	52.3	27.3	0.379	0.018
	b	1	56.4	26.1	0.330	0.014
		2	57.0	24.8	0.323	0.012
HEG 9	a	1	56.6	23.9	0.328	0.011
		2	56.5	23.3	0.329	0.011
	b	1	61.2	31.4	0.274	0.017
		2	59.4	25.7	0.294	0.012
HEG 10	a	1	61.4	24.9	0.270	0.011
		2	65.0	24.6	0.228	0.010
	b	1	62.2	25.8	0.262	0.011
		2	64.5	26.1	0.234	0.011
HEG 11	a	1	66.0	25.2	0.216	0.010
		2	61.9	26.1	0.265	0.011

	b	1	60.3	18.1	0.284	0.007
		2	60.8	17.2	0.278	0.006
HEG 20	a	1	59.4	29.5	0.295	0.016
		2	60.4	28.5	0.283	0.014
	b	1	65.0	26.3	0.228	0.011
		2	60.3	24.4	0.283	0.011
HEG 21	a	1	56.5	bag lost		
		2	52.6	17.0	0.375	0.008
	b	1	52.6	18.2	0.376	0.008
		2	53.0	12.7	0.370	0.005
HEG 26	a	1	56.8	21.4	0.326	0.010
		2	56.3	20.7	0.331	0.009
	b	1	55.1	23.8	0.346	0.012
		2	56.6	19.6	0.328	0.008
HEG 27	a	1	53.7	18.2	0.362	0.008
		2	51.2	20.4	0.392	0.010
	b	1	52.9	21.4	0.372	0.011
		2	48.0	20.6	0.430	0.012
HEG 30	a	1	62.9	29.8	0.253	0.014
		2	61.4	28.5	0.271	0.014
	b	1	61.0	29.1	0.276	0.015
		2	62.6	25.2	0.256	0.011
HEG 37	a	1	55.9	19.1	0.336	0.008
		2	52.6	17.0	0.375	0.008
	b	1	54.9	31.6	0.348	0.024
		2	56.3	27.2	0.331	0.015
HEG 40	a	1	59.6	23.9	0.292	0.010
		2	61.0	26.2	0.275	0.012
	b	1	55.4	33.1	0.342	0.027
		2	55.5	26.4	0.341	0.014
HEG 42	a	1	52.0	20.9	0.383	0.011
		2	51.5	20.9	0.389	0.011
	b	1	54.1	20.7	0.358	0.010
		2	51.5	20.1	0.389	0.010
HEG 43	a	1	57.0	24.1	0.324	0.012
		2	57.1	20.7	0.321	0.009
	b	1	49.8	22.2	0.409	0.013
		2	53.3	21.8	0.367	0.011
HEG 44	a	1	54.2	29.2	0.356	0.019
		2	64.3	16.1	0.237	0.005
	b	1	65.4	16.2	0.223	0.005
		2	56.7	14.9	0.327	0.006
HEG 48	a	1	56.7	25.5	0.327	0.013
		2	60.5	24.2	0.281	0.010
	b	1	58.6	19.6	0.304	0.008
		2	58.7	19.9	0.303	0.008
HEG 50	a	1	53.3	22.5	0.367	0.011
		2	49.4	bag lost		
	b	1	45.1	21.1	0.465	0.014
		2	48.1	23.2	0.428	0.015

Table A.4: Results of the "best" linear mixed model procedure explaining litter mass loss [%] after three months of decomposition on 23 grassland sites with tea type, land-use, soil factors and enzymes as fixed effects and plot as random effect. Estimated effect sizes and lower (LL) and upper (UL) CIs are shown.

Tea mass loss after 3 months [%]	**Estimate**	**LL**	**UL**
(Intercept)	53.855	50.632	57.078
tea type	-33.730	-35.873	-31.588
mowing long	3.154	1.265	5.042
grazing long	1.307	-0.500	3.113
soil dim. 2	-1.015	-1.967	0.064
enzyme dim. 1	0.640	0.183	1.097
enzyme dim. 2	-0.210	-0.804	0.384
tea type x mowing long	-0.812	-2.385	0.761
Observations	46		
Akaike Inf. Crit.	250.3		
R^2m	0.9670		
R^2c	0.9860		

Table contains estimated effects sizes and lower and upper CIs. R^2m = marginal r^2, R^2c = conditional r^2: R^2m accounts for that proportion of variance that is explained by the fixed effects while R^2c accounts for the sum of variance explained by fixed and random effects. Thus, the difference between R^2c and R^2m is a measure of the proportion of variance explained by *plot*. Enzymatic and soil data that were used in PCA (*enzyme dim. 1* and *2*; *soil dim. 1*) were centred and standardized by norm.

Table A.5: Results of the "best" linear mixed model procedure explaining litter mass loss [%] after twelve months of decomposition on 23 grassland sites with tea type, land-use, soil factors and enzymes as fixed effects and plot as random effect. Estimated effect sizes and lower (LL) and upper (UL) CIs are shown.

Tea mass loss after 12 months [%]	**Estimate**	**LL**	**UL**
(Intercept)	63.427	61.001	65.845
Tea type	-29.609	-33.201	-26.017
grazing long	-1.507	-3.601	0.587
soil dim. 1	0.320	-0.601	1.241
soil dim. 2	-0.507	-1.485	0.471
CBH K_m	-1.545	-3.157	0.067
CTH V_{max}	1.956	0.210	3.701
PH V_{max}	1.914	0.373	3.454
Observations	34		
Akaike Inf. Crit.	205.6		
R^2m	0.9456		
R^2c	0.9546		

Table contains estimated effects sizes and lower and upper CIs in parenthesis. R^2m = marginal r^2, R^2c = conditional r^2: R^2m accounts for that proportion of variance that is explained by the fixed effects while R^2c accounts for the sum of variance explained by fixed and random effects. Thus, the difference between R^2c and R^2m is a measure of the proportion of variance explained by *plot*. Soil data that were used in PCA (*soil dim. 1* and *2*) were centred and standardized by norm. Enzymatic data were centred and scaled.

Table A.6: Linear mixed-effects models (nlme package, (Pinheiro et al. 2020)) to find the best set of predictor variables (land-use intensity, enzyme kinetics, basic soil characteristics) that explain tea litter mass loss [%] after three months (n = 46) on 23 grassland sites. Both models were not selected as final models due to significant (ANOVA) higher AIC compared to the "best" model presented in Table 1.

3 months model without grazing					**3 months model without mowing**				
	Df	**t-value**	**F-value**	**p-value**		**df**	**t-value**	**F-value**	**p-value**
tea type	19	-29.80	2483.76	<0.001***	tea type	20	-48.86	2387.19	<0.001***
mowing long	19	2.87	7.42	0.01*	grazing long	19	-0.07	0.16	0.70
soil dim. 1	19	-0.85	1.18	0.30	soil dim. 1	19	0.61	0.27	0.61
soil dim. 2	19	-1.72	2.38	0.14	soil dim. 2	19	-2.11	3.57	0.07
enzyme dim. 1	19	2.54	6.01	0.02*	enzyme dim. 1	20	2.24	4.97	0.03*
enzyme dim. 2	19	-0.67	0.19	0.67	enzyme dim. 2	20	-0.74	0.56	0.46
tea type x mowing long	19	-0.97	0.94	0.35					
AIC	251.60				AIC	263.45			
	marginal	**conditional**				**marginal**	**conditional**		
R^2	0.9656	0.9859			R^2	0.95499	0.9849		

Results of linear mixed effects model, analysis of variance (ANOVA) and pseudo-R-squares of generalized mixed-effects model. *** < 0.001, ** < 0.01, * < 0.05. Marginal R^2 is for fixed effects only, conditional R^2 also includes the random effect *plot*.

Litter quality is expressed by tea type, green tea (C/N ratio: 12.5) and rooibos tea (C/N ratio: 66.9). *mowing long* and *grazing long* characterize land-use intensity and were calculated for the time period 2006 to 2016 (three-month model) (Blüthgen et al., 2012, Vogt et al., 2019). *Soil dim.1* and *soil dim.2* represent the first and second axis of a dimensional extraction using PCA. *Soil dim.1* contains information on the nutrient situation and *soil dim.2* on the soil microbial conditions. The variables *enzyme dim.1* and *enzyme dim.2* also derive from another PCA, where *enzyme dim.1* comprises the V_{max} values and *enzyme dim.2* the K_m values of the five investigated EHEs.

Table A.7: Linear mixed-effects models (nlme package, (Pinheiro et al. 2020)) to find the best set of predictor variables (land-use intensity, enzyme kinetics, basic soil characteristics) that explain tea litter mass loss [%] after 12 months (n = 34) on 23 grassland sites. Both models were not selected as final models due to significant (ANOVA) higher AIC compared to the “best” model presented in Table 1.

12 months with BG K_m, but without CBH K_m					**12 months without CBH K_m**				
	Df	**t-value**	**F-value**	**p-value**		**df**	**t-value**	**F-value**	**p-value**
tea type	9	-9.36	387.43	<0.001***	tea type	10	-15.55	395.85	<0.001***
grazing long	17	-1.46	6.34	0.02*	grazing long	17	-1.68	6.45	0.02*
soil dim. 1	17	0.15	0.31	0.59	soil dim. 1	17	0.17	0.31	0.58
soil dim. 2	17	-0.95	0.20	0.66	soil dim. 2	17	-0.97	0.21	0.67
BG K_m	9	0.65	0.87	0.37	CTH V_{max}	10	2.47	5.77	0.04*
CTH V_{max}	9	2.46	5.69	0.04*	PH V_{max}	10	2.17	4.71	0.06*
PH V_{max}	9	2.03	4.12	0.07					
AIC	208.61				AIC	207.17			
	marginal	**conditional**				**marginal**	**conditional**		
R^2	0.9413	0.9413			R^2	0.9404	0.9404		

Results of linear mixed effects model, analysis of variance (ANOVA) and pseudo-R-squares of generalized mixed-effects model. *** < 0.001, ** < 0.01, * < 0.05. Marginal R^2 is for fixed effects only, conditional R^2 also includes the random effect *plot*.

Litter quality is expressed by tea type, green tea (C/N ratio: 12.5) and rooibos tea (C/N ratio: 66.9). *mowing long* and *grazing long* characterize land-use intensity and were calculated for the time period 2006 to 2017 (12-month model) (Blüthgen et al., 2012, Vogt et al., 2019). *Soil dim.1* and *soil dim.2* represent the first and second axis of a dimensional extraction using PCA. *Soil dim.1* contains information on the nutrient situation and *soil dim.2* on the soil microbial conditions. The individual enzymes were included in the model (K_m of β-glucosidase (*BG K_m*), K_m of cellobiohydrolase (*CBH K_m*), V_{max} of cellotriohydrolase (*CTH V_{max}*), V_{max} of phosphatase (*PH V_{max}*).