
Supplementary material for the article

Smallholder farmers' practices and African Indigenous Vegetables affect soil microbial biodiversity and enzyme activities in Lake Naivasha Basin, Kenya

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Table S1. PCR reaction mixtures and thermal profiles for different target genes used.

Target Gene	Reaction Mix	Volume (μL)	Step 1
16s rRNA 1 st step	Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 5 min 25x { 94°C 30s 50°C 30s 72°C 30s 72 °C 10 min.
	Nuclease free water	8	
	DNA template (1ng/μL)	2	
	Primer 343F (10μM)	1.25	
	(5'-TACGGRAGGCAGCAG-3')		
	Primer 802R (10μM)	1.25	
	(5'-TACNVGGGTWTCTAATCC-3')		
Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 5min 10x { 95°C 30s 50°C 30s 30°C 30s 72 °C 10min.	
Nuclease free water	8		
1st Step Amplicons	1.25		
Primer 343F (10μM)	1.25		
(5'-TACGGRAGGCAGCAG-3')			
Primer 802R (10μM)	1.25		
(5'-TACNVGGGTWTCTAATCC-3')			
ITS 1 st step	Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 4 min 28x { 94°C 30s 56°C 30s 72°C 1min 72 °C 7min
	Nuclease free water	8	
	DNA template (1ng/μL)	1.25	
	Primer ITS-1 (10μM)	1.25	
	(5'-TCCGTAGGTGAACCTGCGG-3')		
	Primer ITS-2 (10μM)	1.25	
	(5'-GCTGCGTTCTTCATCGATGC-3')		
ITS 2 nd step	Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 4 min 7x { 94°C 30s 56°C 30s 72°C 1min 72 °C 7min
	Nuclease free water	8	
	1 st Step Amplicons	1.25	
	Primer ITS-1 (10μM)	1.25	
	(5'-TCCGTAGGTGAACCTGCGG-3')		
	Primer ITS-2 (10μM)	1.25	
	(5'-GCTGCGTTCTTCATCGATGC-3')		

PCR products generated from the second step were multiplexed as a single pool, separately for ITS and 16s amplicons and the pool was purified using Agencourt AMPure XP kit (REF A63880, Beckman Coulter, Milan, Italy) according to the manufacturer's protocol.

Table S2. Detailed methodology used for enzymatic assays of soil samples.

En- zyme	Incubated In	Incubation Conditions	Reaction Stopped Fol- lowing Incubation by Adding	Activity Determined by
β - GLU	Buffered substrate solution (Modified Universal Buffer MUB pH 6.0 + 25mM 4-Ni- trophenyl β -D-glucopyra- noside)	Continuous shaking 250rpm, 37°C, 1h.	0.1M Tris-(hydroxylme- thyl)-aminomethane pH 12 and 0.5M CaCl ₂ and vigorous shaking	Immediate separation of liquid phase and then spectrophotometrically at 405nm of liq- uid phase
PHO	Buffered substrate solution (MUB pH 6.5 + 25 mM p- nitrophenyl phosphatase)	Continuous shaking 250rpm, 37°C, 1h.	0.5M NaOH and 0.5M CaCl ₂ and vigorous shaking	
URE	Buffered substrate solution (Boric Buffer, pH 10 + 0.72M UREA)	Continuous shaking 250rpm, 37°C, 2h.	1N KCl/0.01N HCl solu- tion and vigorous shak- ing	Liquid phase separated, then mixed with So- dium Salicylate solution (equal mix of 0.12% Na ₂ Fe(CN) ₅ NO, of 17% C ₇ H ₅ NaO ₃ and H ₂ OBD) and 1mL of 0.1% C ₃ Cl ₂ N ₃ NaO ₃ . Re-incubated at 24°C, 30 min and then spec- trophotometrically determined at 690nm

Table S3. Physicochemical parameters of each surveyed field.

	TOC (%)	N (%)	P _{act} (mg/kg)	K _{act} (meq/100g)	pH (H ₂ O)	pH (KCl)	CEC (cmol/kg)	EC (ds/m)
F1	2.66 (±0.19)*	0.23 (±0.01)	131 (±13.86)	2.37 (±0.12)	6.4 (±0.19)	5.52 (±0.11)	8.9 (±0.4)	0.08 (±0.03)
F1.2	2.06 (±0.15)	0.18 (±0.01)	100.08 (±8.27)	3.38 (±0.12)	6.6 (±0.15)	5.55 (±0.05)	8.3 (±0.8)	0.1 (±0.01)
F2	1.8 (±0.13)	0.16 (±0.01)	77.84 (±2.28)	3.63 (±0.28)	6.8 (±0.05)	5.37 (±0.08)	7.3 (±0.5)	0.05 (±0.01)
F3	2.36 (±0.13)	0.19 (±0.01)	101.47 (±9.73)	4.49 (±0.17)	6.5 (±0.04)	5.45 (±0.03)	6 (±0.7)	0.1 (±0)
F4	1.56 (±0.03)	0.13 (±0)	82.35 (±1.59)	4.65 (±0.3)	6.6 (±0.06)	5.13 (±0.02)	8.5 (±0.5)	0.08 (±0)
F5	1.43 (±0.14)	0.15 (±0)	288.07 (±29.8)	7.53 (±0.28)	8.7 (±0.05)	7.31 (±0.06)	5.6 (±0.6)	0.17 (±0.01)
F6	2.55 (±1.09)	0.17 (±0.02)	142.82 (±20.51)	8.7 (±0.19)	8.9 (±0.06)	7.48 (±0.09)	7.4 (±1.1)	0.19 (±0.02)
F7	1.67 (±0.2)	0.17 (±0.01)	137.95 (±11.57)	9.52 (±0.31)	8.7 (±0.06)	7.37 (±0.05)	11.9 (±0.4)	0.28 (±0.02)
F8	2.11 (±0.22)	0.22 (±0)	161.93 (±20.25)	11.23 (±0.69)	8.7 (±0.07)	7.28 (±0.03)	11.5 (±2.6)	0.33 (±0.03)
F9	1.16 (±0.08)	0.11 (±0.01)	283.9 (±5.85)	8.21 (±0.29)	8.9 (±0.09)	7.75 (±0.04)	12.5 (±0.3)	0.2 (±0.03)
F10	3.29 (±0.21)	0.21 (±0.02)	87.91 (±6.26)	3.97 (±0.54)	7.0 (±0.21)	6.28 (±0.31)	15.7 (±1.4)	0.6 (±0.11)
F12	4.76 (±0.38)	0.32 (±0.02)	268.95 (±41.91)	17.8 (±3.32)	7.6 (±0.12)	7.13 (±0.11)	10.4 (±2.1)	0.93 (±0.18)
SSN1	2.07 (±0.17)	0.12 (±0.01)	141.77 (±26.6)	5.85 (±1.35)	7.2 (±0.08)	5.97 (±0.13)	14.2 (±3.1)	0.38 (±0.07)
SSN2	2.48 (±0.05)	0.17 (±0)	177.91 (±9.13)	7.2 (±0.4)	7.3 (±0.18)	6.32 (±0.25)	18.4 (±0.7)	0.27 (±0.01)
SSN3	2.4 (±0.11)	0.22 (±0.01)	139.69 (±6.94)	8.21 (±0.12)	8.0 (±0.07)	6.7 (±0.06)	17.8 (±1.9)	0.22 (±0.01)

*(std error of the means)