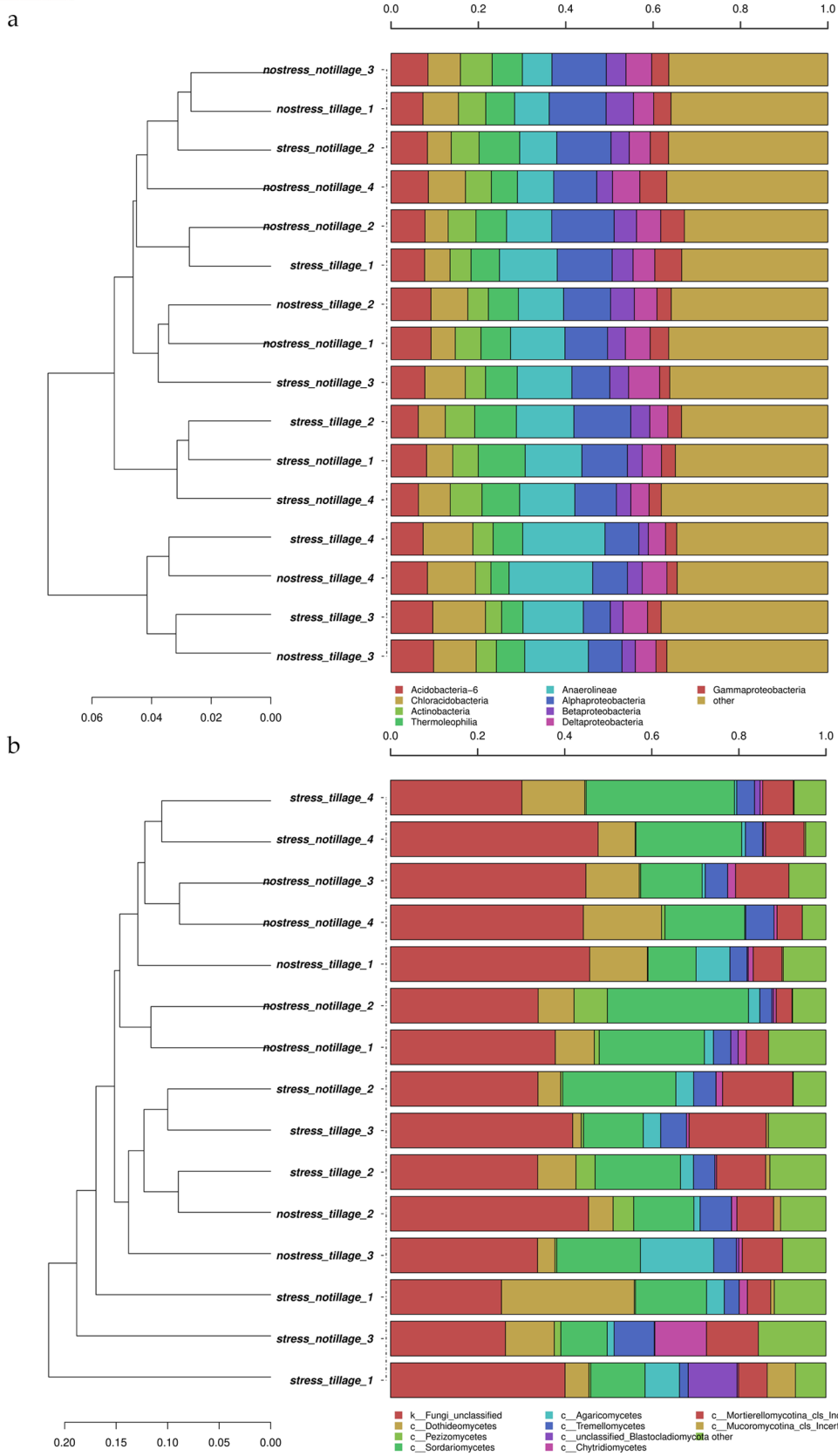


Supporting Information for the Article

Combined impact of no-till and cover crops with *or* without short-term water stress as revealed by physico-chemical and microbiological indicators

Eren Taskin¹, Roberta Boselli², Andrea Fiorini², Chiara Misci¹, Federico Ardeni², Francesca Bandini¹, Lorenzo Guzzetti³, Davide Panzeri³, Nicola Tommasi³, Andrea Galimberti³, Massimo Labra³, Vincenzo Tabaglio^{2*}, Edoardo Puglisi¹,

* Correspondence: vincenzo.tabaglio@unicatt.it ; +39-0523-599-222 (V.T.)



Supplementary Figure 1

Supplementary Table 1. PCR reaction mixtures and thermal profiles for different target genes used

Target Gene	Reaction Mix	Volume (μL)	Step 1
Bacterial 16s 1 st step	Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 5 min 20x { 94°C 30s 50°C 30s 72°C 30s 72 °C 10 min.
	Nuclease free water	8	
	DNA template (1ng/ μL)	2	
	Primer 343F (10 μM) (5'-TACGGRAGGCAGCAG-3')	1.25	
	Primer 802R (10 μM) (5'-TACNVGGGTWTCTAATCC-3')	1.25	
Bacterial 16s 2 nd step	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 (10 μM) (5'-TACGGRAGGCAGCAG-3')	1.25	
	Primer 802R (10 μM) (5'-TACNVGGGTWTCTAATCC-3')	1.25	
Fungal ITS 1 st step	Phusion Flash High-Fidelity Master Mix	12.5	94 °C - 4 min 25x { 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 (10 μM) (5'-TCCGTAGGTGAACCTGCGG-3')	1.25	
	Primer ITS-2 (10 μM) (5'-GCTGCGTTCTTCATCGATGC-3')	1.25	
Fungal 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 (10 μM) (5'-TCCGTAGGTGAACCTGCGG-3')	1.25	
	Primer ITS-2 (10 μM) (5'-GCTGCGTTCTTCATCGATGC-3')	1.25	

Supplementary Table 2. Detailed methodology used for enzymatic assays of soil samples.

Enzyme	Incubated In	Incubation Conditions	Reaction Stopped Following Incubation by Adding	Activity Determined by
β -GLU	Buffered substrate solution (Modified Universal Buffer MUB pH 6.0 + 25mM 4-Nitrophenyl β -D-glucopyranoside)	Continuous shaking 250rpm, 37°C, 1h.	0.1M Tris-(hydroxymethyl)-aminomethane pH 12 and 0.5M CaCl ₂ and vigorous shaking	Immediate separation of liquid phase and then spectrophotometrically at 405nm of liquid 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	Liquid phase separated, then mixed with Sodium 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 spectrophotometrically determined at 690nm
URE	Buffered substrate solution (Boric Buffer, pH 10 + 0.72M UREA)	Continuous shaking 250rpm, 37°C, 2h.	1N KCl/0.01N HCl solution and vigorous shaking	