

Table 1. Overview of the soil physical, chemical, and microbial properties evaluated. Please see text for further details.

Soil Properties	Method description	Samples or sites evaluated
<b>Physical Properties</b>		
Bulk Density	InstroTek 3500 Xplorer Nuclear Moisture Density Gauge (Las Vegas, NV) at 5 and 20 cm.	Determined at the CTN1, CTN2, FRG_CTN, OWB_BER, and OWB_RC. Not measured on the OWB_ROT and CTN3 agroecosystems because 1997 data were not available
Particle size analysis	Combination of sieving and sedimentation techniques as described by Kettler et al (2001).	All agroecosystems for all samples
Water-stable aggregate isolation	Four water stable aggregate fractions were obtained from a wet-sieving method of air-dried soils Elliott (1986). The four fractions were: large macroaggregates (> 250 µm), small macroaggregates (> 250 µm), microaggregates (53-250 µm), and silt plus clay (< 53 µm).	All agroecosystems for all samples
Mean weight diameter	Data from the water stable aggregate fractionation were used to calculate mean weight diameter (an indicator of aggregate stability) based on the proportion of the soils with each of the water stable aggregate fractions. Calculations were done using the following equation: $MWD = \sum PiDi$ (van Bavel, 1949). Pi is the proportion of the whole soil in the given fraction, Di is the average diameter (mm) of the particles of the fraction.	All agroecosystems for all samples.
Intra-aggregate isolation	Three intra-aggregate fractions were isolated from the macroaggregate fraction (Six et al. 2000): intra-aggregate particulate organic matter (> 250 µm), intra-aggregate microaggregates (53-250 µm), and intra-aggregate silt plus clay (< 53 µm).	All agroecosystems
<b>Chemical Properties</b>		
Total organic C and N	Dry combustion analysis using a LECO TruSpec CN Analyzer (St. Joseph, MI). No detectable inorganic C was measured (Non-carbonate Carbon Method; LECO); thus measured total C represents soil organic C.	All agroecosystems; included grazing exclusions
pH	1:1 soil to water ratio ; Ward Lab; Kearney, NE	All agroecosystems; included grazing exclusions
Organic matter	Loss on ignition at 400 degrees C; Ward Lab; Kearney, NE	All agroecosystems; included grazing exclusions
<b>Greenhouse gas fluxes</b>		
CO <sub>2</sub>	Gas sampling occurred between 0800h and 1300h hours on all sampling dates using a LI-COR LI-8100a (Lincoln,NE) with a 20 cm survey chamber system interfaced with a Theta moisture (Dynamax; Houston, TX) and temperature probe.	2-3 collars installed in each replicate of PNG, both crops of millet-cotton rotation (all within FRG_CTN), and BER and OWB2 (within OWB_BER).
N <sub>2</sub> O	Static chamber system (Hutchinson and Mosier, 1981) was designed to allow for gas collection from the same collars used for CO <sub>2</sub> flux measurements. 30 mL samples were drawn at 15 minute intervals for 45 minutes. Gas samples were stored in syringes maintained under pressure and analyzed within 36 hours using a Shimadzu GC-2013 gas chromatograph (Kyoto, Japan). Additionally, soil temperature and volumetric soil moisture using a Campbell Scientific Hydrosense probe (Logan, UT) were collected at 10 cm depth for each sampling time.	2-3 collars installed in each replicate of PNG, both crops of millet-cotton rotation (all within FRG_CTN), and BER and OWB2 (within OWB_BER).
<b>Microbial Properties</b>		
Microbial biomass C and N	Microbial biomass C (MBC) and N (MBN) were assessed via the chloroform-fumigation extraction method (Brookes et al., 1985; Vance et al., 1987).	CTN1, CTN2, FRG_CTN, OWB_BER, and OWB_RC for 0-5 and 5-20cm
Microbial Composition	Ester-linked fatty acid methyl esters (EL-FAMES) profiling according to the method by (Schutter and Dick, 2000).	CTN1, CTN2, FRG_CTN, OWB_BER, and OWB_RC for 0-5 and 5-20cm
Enzyme activities	C-cycling enzymes (β-glucosidase, α-galactosidase, and β-glucosaminidase), P-cycling (alkaline phosphatase and phosphodiesterase) and S-cycling (arylsulfatase) were assayed using the colorimetric methods with p-nitrophenyl derivate substrate analogues as described in Tabatabai (1994) and Parham and Deng (2000).	CTN1, CTN2, FRG_CTN, OWB_BER, and OWB_RC for 0-5 and 5-20cm
DNA extraction and pyrosequencing analyses	DNA was extracted from 0.7 g of moist soil using the Fast DNA Spin Kit for soil (MP Biomedicals, OH, USA) according to the manufacturer's instructions. DNA was submitted to the Research and Testing Lab (Lubbock, TX) for bacterial and fungal sequencing on a 70x75 GS PicoTiterPlate (PTP) via Titanium sequencing platform (Roche, Nutley, New Jersey).	CTN1, CTN2, FRG_CTN (PNG and combined DNA from both phases of the CTN-Mi rotation), OWB_BER (only one of the OWB paddocks plus BER), and OWB_RC (only one of the OWB paddocks plus the corn) for whole soil and water stable aggregates from 0-5 cm.
Fungal C utilization profiles	Assessed via the FungiLog procedure, as described by (Dobranic and Zak, 1999) Dobranic and Zak (1999), which uses Biolog SFN2 96-well microtiter plates containing 95 different C substrates (Biolog, Hayward, CA, USA).	CTN1, CTN2, FRG_CTN, OWB_BER, and OWB_RC for 0-5 and 5-20cm; included grazing exclusions