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Supplement of

Smallholder farms in eastern African tropical highlands have low soil greenhouse gas fluxes

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1.1 Methods

A soil core incubation study was conducted to compare the effects of the different land-classes, field types and cover types on potential soil GHG fluxes; and to test if potentials of soil GHG fluxes under standardized conditions in the laboratory mirror differences in annual GHG fluxes at observation sites. Five soil cores were collected from 36 out of 59 plots using a 5 cm long PVC pipe (5.14 cm ID). The cores were left intact and taken back to the lab where they were air-dried (2 d at 30°C). One core from each plot was soaked overnight in water and then freely drained for 2-3 hours and then oven-dried (24h at 105°C) to determine maximum water-holding capacity (WHC). Three replicates of the air dried cores for each plot were then placed into a self-sealing 0.50 L glass jar fitted with a septum at 20°C. Air samples (10 mL) from each jar were collected at 0, 15, 30 and 45 min. The air samples were analyzed immediately for CO₂, CH₄ and N₂O in an SRI 8610C gas chromatograph (9' Hayesep D column) fitted with a ⁶³Ni-electron capture detector for N₂O and a flame ionization detector for CH₄ and CO₂ (after passing the CO₂ through a methanizer). The flow rate for the carrier gas (N₂) was 20 mL min⁻¹. Every fifth sample analyzed on the gas chromatograph was a calibration gas (gases with known CO₂, CH₄ and N₂O concentrations in synthetic air) and the relation between the peak area from the calibration gas and its concentration was used to determine the CO₂, CH₄ and N₂O concentrations of the headspace samples. The soil cores were then brought to 25% WHC, left for one hour and then placed in the same jar and the headspace was again sampled and analyzed as above. This was sequentially repeated for the same cores at 35, 55 and 75% WHC. Soil re-wetting is known to result in a flush of nutrients (Birch, 1960) that tends to diminish with subsequent re-wettings. Therefore, for the subsequent re-wettings we also added a dilute KNO₃ solution (equivalent to adding 10 mg N kg⁻¹ soil) to replace the N lost.

The flux rates for CH₄, CO₂ and N₂O were compared using ANOVA (AOV in RStudio v. 0.98.953), using the WHC as blocks and cover type, land class, and field type as fixed

factors. Because of the imbalanced design, we could not analyze interactions as several combinations had an insufficient number of samples so each of the factors was analyzed independently of the others. When $P < 0.1$, differences between treatments were analyzed using Tukey's HSD. Correlations between maximum flux rates for the intact soil core incubations and total cumulative fluxes for the field measurements were tested using Spearman Rank Correlation.

1.2 Results

For the laboratory incubations, there was very little CO₂ efflux (maximum of 7.5 mg CO₂-C m⁻² h⁻¹) when the soils were air-dried, with increased soil respiration only at higher water contents (Fig. S1). For the five investigated soil moisture levels (air dried, 25, 35, 55 and 75% WHC) soil respiration tended to be highest at 55% WHC (Figs. 2, 3 and 4) and was positively correlated with the soil C and N content ($r=0.33$, $P = 0.005$ and $r=0.35$, $P = 0.003$ respectively). The N₂O fluxes were very low when the water content was less than or equal to 35% WHC and increased exponentially when the water content was increased to 55 and 75% (Fig. S1) and were also positively correlated with total C and N ($r = 0.24$, $P = 0.043$ and $r = 0.31$, $P = 0.010$ respectively). The soil CH₄ fluxes (mostly uptake) were generally low, ranging from -20 to 20 µg CH₄-C m⁻² h⁻¹ and unlike the previous two GHGs, there were similar flux rates between the three moderate water contents, while there were much lower fluxes at the lowest and highest water contents (Fig S1). Unlike N₂O and CO₂ fluxes, CH₄ fluxes were not correlated with soil C and N contents.

Both the CO₂ and the N₂O fluxes differed by land class ($P = 0.001$ and 0.061 respectively) with land class 1 (lowland farms with degraded soils) having lower CO₂ fluxes than classes 4 (mid-slope farms and shrub land) and 5 (lowland pasture), while landclass 4 had higher N₂O fluxes than either class 1 or 2 (highland farms) (Fig. 2). As shown in Table 2, land class 1 and 2 also had the lowest soil C and N contents. Grass and grazing plots emitted more CO₂ than annual plots ($P = 0.069$), while there were no detectable differences in N₂O or CH₄ fluxes between vegetation types ($P = 0.603$ and 0.457 respectively). Field type had no detectable difference on CO₂, N₂O or CH₄ fluxes ($P = 0.179$, 0.109 , and 0.198 respectively).

A Spearman rank correlation between maximum N₂O fluxes observed within the soil core study and the cumulative field emissions showed a strong correlation ($\rho = 0.399, P = 0.040$), while CO₂ fluxes followed a similar trend ($\rho = 0.349, P = 0.075$). The CH₄ fluxes from the soil cores were not however, correlated with measured flux at the field sites ($\rho = -0.145, P = 0.471$).

Fig. S1. CO₂ (mg C- CO₂ m⁻² h⁻¹), CH₄ (μg C- CH₄ m⁻² h⁻¹), and N₂O (μg N₂O-N m⁻² h⁻¹) flux rates from intact soil cores taken from 36 sites across 5 different land classes (Land class 1 = degraded lowland farms; class 2 = degraded farms, lower slopes; class 3 = mid slopes, grazing; class 4 = upper slopes/plateau, mixed farms; and class 5 = mid slopes moderate sized farms) in western Kenya incubated at 20°C and 5 different water content (0 [air dried], 25, 35, 55, and 75% WHC). Different lower case letters following the land class number indicate differences between treatments (i.e. land classes)



