The effect of tillage management on microbial functions in a maize crop at different slope positions

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Abstract

Determining whether agricultural soils act as sinks or sources of greenhouse gases (GHGs) requires the quantification of variations in the pools and fluxes of soil organic carbon (SOC) and nutrients, e.g. nitrogen (N), as well as the associated soil microbial responses. In this study, soil was collected from experimental plots of maize (*Zea mays*) under conventional, minimum or strip tillage treatments established in a sloping field (~10%) of loamy soil in SW England, UK, where maize had been cultivated conventionally for 12 years. Topsoil (0–10 cm) cores were collected from the top, mid, bottom and foot slope positions to investigate soil C, N and microbial properties. The impact of conventional management on potential GHG emissions was assessed by incubating soils collected from the top, mid and bottom slope positions from the conventional treatment. Contents of SOC and total N were greatest at the top slope position and soil mineral N (NO_3^--N and NH_4^+-N) concentrations were greater at the bottom and foot slope positions in all treatments. Biomarker phospholipid fatty acids (PLFA) for Gram positive bacteria and fungi were relatively ^{13}C-enriched at each slope position regardless of treatment, indicating preferential utilization of organic matter from maize (C_4) rather than SOC (C_3). Around 70% of carbon incorporated into total PLFA was derived from C_3-SOC at the slope foot, indicating that more SOC older than 12 years was being mineralized at the depositional position. Effluxes of N_2O and CO_2, and total GHG emission were greatest from the incubated soils sampled from the bottom slope position, suggesting that conditions in depositional positions of regularly ploughed sloping arable fields may have increased the potential for mineralization and denitrification. We conclude that the C sink potential of the depositional positions of slopes may be diminished by coincidental GHG emissions.

Keywords: Soil tillage; Carbon and nitrogen cycling; Greenhouse gas emissions; Soil microbial community; Compound-specific ^{13}C stable isotope analysis

1 Introduction

A substantial proportion of land under conventional arable cultivation is degraded, and losses of soil by erosion can be significant in sloping land (Lal, 2003; Van Oost et al., 2007). The downslope transport and deposition of soil affects the physical distribution of sediment-associated organic carbon (C) (Zhang et al., 2006) and alters the process of C mineralization (Gregorich et al., 1998), influencing the exchange of C between the pedosphere and the atmosphere (Harden et al., 1999; Lal, 2003). Quantifying the strength of agricultural soil erosion as a global C flux is much debated, with markedly different estimates ranging from a net source of 0.4 to 1.2 Pg C a^{-1} (Jacinthe and Lal, 2001; Lal et al., 2004; Lal and Pimental, 2008) to a net sink of 0.1 to 1.0 Pg C a^{-1} (Smith et al., 2001, 2005; Van Oost et al., 2007).

Transfer of soil from higher to lower positions in the slope can be beneficial if C remains at the bottom of the slope, which increases the potential for C accumulation (Lal, 2018). This, however, can also be a risk as other processes can counteract this benefit due to gaseous losses of other nutrients that represent a bigger component of the greenhouse gas balance (Henault et al., 2012). Emissions of carbon dioxide (CO_2) have been measured in eroding agricultural slopes (Van Hemelryck et al., 2011) but there are few studies exploring emissions of nitrous oxide (N_2O) from the same soils. Indeed, the role of erosion in soil N dynamics has received comparatively little attention (Berhe and Torn, 2017). Furthermore, the total GHG balance which includes methane (CH_4) emissions is missing. This GHG is 25 times more powerful than CO_2 and can be taken up in soils but there is a potential for production under high soil moisture contents (Oertel et al., 2016).

Lateral fluxes of nitrogen (N) from sloping agricultural land can be similar in magnitude to those from fertilizer application and crop removal (Quinton et al., 2010). N availability has a profound influence on the functioning of soil microorganisms (Dungait et al., 2012), which are the main biotic drivers of soil C efflux (Paterson et al., 2008). Nitrogen dioxide (N_2O) emissions from agriculture can contribute up to 2.8 Pg CO_2-eq a^{-1} (Smith et al., 2007). As the global warming potential of N_2O is 298 times relative to that of CO_2 (IPCC, 2013), the potential of soil organic carbon (SOC) burial at the bottom of eroding slopes to act as a small C sink could easily be negated by a coincidental increase in N_2O emissions. The importance of soil position in the landscape on N_2O emissions has been recognized (Vilain et al., 2012; Han et al., 2017; Saha et al., 2017; Singh et al., 2019) and attributed to the influence of topography on hydrological and pedological processes that regulate soil water content, microbial biomass, N mineralization, nitrification and denitrification (Sehy et al., 2003; Florinsky et al., 2004). Compared to the variability observed in field measurements of N_2O, laboratory incubations are considered to provide reliable results for estimating the potential influence of soil C dynamics on N_2O emissions under controlled conditions of temperature and moisture (Schaufler et al., 2010).

The production of N_2O derives from nitrification in dry or well-aerated soils, or incomplete denitrification at medium-to-high soil moisture conditions (Baggs et al., 2000). Both N_2O-producing processes are strongly affected by climate, SOC content, soil texture, soil drainage, availability of N (NO_3^--N and NH_4^+-N) and management-related factors (Snyder et al., 2009) which are all changed during soil erosion. Decreasing C availability to soil microorganisms through physical protection of C after burial at depositional slope positions may reduce N_2O emissions (Harden et al., 1999). However, soil moisture content is likely to be persistently greater at the bottom of slopes, increasing the potential for high water-filled pore space (WFPS) that promotes denitrification (Lal and Pimental, 2008). Moreover, the enhanced availability of biologically available C at the bottom of slopes due to selective transport of light fraction (Zhang et al., 2006), slaking of aggregates (Lal, 2003), and leaching of soluble C and NO_3^- may promote conditions favorable for N_2O production. However, increasing C availability may also encourage the reduction of N_2O to N_2 thereby reducing N_2O emissions (Sánchez-Martín et al., 2008; García-Marco et al., 2014).

The responses of soil microorganisms to tillage management are important for regulating GHG emissions from sloping farmland. A previous study in a sloping arable soil under conventional tillage (i.e. annual ploughing) found that soil microorganisms from different slope positions presented contrasting responses to erosion-induced redistribution of SOC after two years of C4 maize cultivation, with relative ^{13}C-enrichment of soil microbial biomass C in the eroding top slope (Dungait et al., 2013a). Whether this was a general response of the whole soil microbial community or specific to different microbial groups can be revealed by using phospholipid fatty acids

(PLFA) analysis, a commonly used method of measuring the abundance and composition of major microbial groups (Frostegård et al., 2011). In a system where C4 crops like maize are grown in a C3 soil (i.e. where only C3 plants have grown previously), natural abundance ^{13}C-PLFA analysis can be used to determine the uptake of different sources of carbon by broad functional groups of soil microorganisms (e.g. Yao et al., 2015).

In this study, we tested the hypothesis that soil conditions in downslope positions in temperate arable fields promote GHG emissions (CO_2, CH_4 and N_2O). We used an existing experiment where contrasting conventional, minimum or strip tillage managements had been established in a field site with erosion-susceptible soils typically used for maize production in SW England, UK, to explore opportunities to manage tillage erosion (Norris, 2015). The study comprised 2 aspects: (i) determine the differences between the soil properties (soil moisture content, available C and N) and soil microbial activity (using CO_2 efflux measurements and compound-specific ^{13}C-PLFA analyses) at different slope positions under different tillage management; (ii) investigate the potential for denitrification at different slope positions under conventional management using an incubation study.

2 Materials and methods

2.1 Experimental site description

Full details of the experimental site are given in Norris et al. (2016). In brief, experimental plots (10 m × 60 m, n = 3; see Fig. S1 in the Supplementary material) of maize (*Zea mays*) were established in June 2012 on a freely draining, slightly acidic loam soil (Eutric Chromic Endoleptic Cambisol; IUSS WRB, 2015) in a sloping field (ca. 10%) in Devon, SW England, UK (N 50°80′04″, W 03°79′34″). The mean annual temperature and precipitation were 10 °C and 1048 mm, respectively. The initial soil properties (June 2012) for the 0–15 cm depth were pH 7.3_{water}, SOC 1.2%, P index 5, K index 3, Mg index 2. The entire field was changed from conventional arable cropping (C3) to conventional maize (C4) cultivation in 2002. The plots were established under three different cultivation treatments: (1) *plough*: conventional plough-based cultivation (mouldboard inversion plough; 20 cm depth); (2) min-*till*: non-inversion cultivation; (3) *strip-till*: maize undersown with perennial ryegrass (*Lolium perenne*), where only the crop row area was ploughed and the maize drilled directly into this area; with three replicates of each treatment (hereafter called 'management'). Urea (100 kg N ha^{-1}) and monoammonium phosphate (20 kg N ha^{-1}) were applied annually in early May.

2.2 Soil sampling and analysis

Soil was sampled in late July 2014 from top, mid, bottom and foot positions (the former three are part of the slope, and the foot is on the flat area after the bottom position; Fig. S2) along the slope transect of the three maize management treatments. The weather immediately prior to soil sampling was warm and dry, with no large rainfall events for several weeks beforehand (Fig. S3). Five cores of surface soil samples (0–10 cm depth × 4 cm diameter) were collected at three points at each slope position and composited, yielding three soil samples per slope position for each management treatment. Sub-samples (10 g) of fresh soil from each field sample were weighed and oven-dried to constant weight at 105 °C for moisture determination. Nitrate-N and NH_4^+-N were extracted from fresh, sieved (2 mm) soil samples using 2 M KCl and filtered through filter papers (2 V, GE Whatman, Little Chalfont, UK) for analysis using a continuous flow analyser (SKALAR SAN^{Plus}, Breda, The Netherlands). Sub-samples for measuring %SOC and %TN, δ^{13}C and δ^{15}N values were oven-dried (60 °C), ball-milled and decarbonated (with 1 M HCl) and then determined using an elemental analyser (Carlo Erba NA2000, CE Instruments, Wigan, UK) interfaced to an isotope ratio mass spectrometer (20–20, SerCon Ltd, Crewe, UK). δ^{13}C and δ^{15}N values were expressed relative to the international PDB and AIR standards, respectively. The analytical precisions of the δ^{13}C and δ^{15}N measurements were < 0.1‰ and < 0.2‰, respectively. Soil pH was measured in air-dried soil with a Jenway 3320 pH meter (soil:water ratio 1:2.5, w/v).

2.3 ^{13}C-PLFA analysis

Phospholipid fatty acids (PLFAs) were extracted from freeze-dried, ball-milled soil samples (sub-samples from the above) using a modified Bligh Dyer extraction procedure (Dungait et al., 2010). In brief, Bligh Dyer solvent was used to extract a total lipid extract which was fractioned into neutral lipids, glycolipids and phospholipids using solid phase extraction. Nonadecane in hexane (0.1 mg ml^{-1}) was added to the phospholipid fraction as the internal standard. The phospholipid fraction was saponified and PLFAs were extracted with hexane and methylated using dry acidified methanol. The PLFA methyl esters were analyzed by gas chromatography (GC) using a Hewlett Packard 5890 Series II GC equipped with a Varian VF23ms column (Varian Ltd., Oxford, UK); GC-mass spectrometry (GC–MS) using a Finnigan Trace GC–MS (ThermoFinnigan: Hemel Hempstead, UK) and, GC-combustion-isotope ratio MS (GC-C-IRMS) analyses using a Varian 3400 GC attached to a Finnigan MAT Delta S isotope ratio MS. Samples were run in triplicate and were calibrated against reference CO_2 of known isotopic composition, which was introduced directly into the source three times at the beginning and end of every run.

Biomarker PLFA for taxonomic groups of microorganisms were identified using GC–MS, and interpreted according to Frostegård and Bååth (1996): the PLFAs indicating bacterial biomass were *i*15:0, *a*15:0, *i*16:0, 16:1ω9, 16:1ω7c, 10Me16:0, cy17:0, *i*17:0, *a*17:0, 18:1ω7, and cy19:0; Gram positive bacteria were represented by bacteria branched-chained *iso*/*anteiso* fatty acids; Gram negative bacteria by monounsaturated and cyclic fatty acids; Actinobacteria by 10Me-branched fatty acids; and, fungi by octadecadienoic acid (18:2ω6). The natural abundance δ^{13}C values of the vegetation at the site were taken from Norris et al. (2016) and used as end-member values. The proportion of C (f) that was derived from C_3-C (ryegrass; δ^{13}C = -29.3‰) or C_4-C (maize; δ^{13}C −12.2‰) and incorporated into individual PLFA was calculated using the expression (Equation 1):

$$fC_3 = \frac{\delta^{13}C_{PLFA} - \delta^{13}C_{C4}}{\delta^{13}C_{C3} - \delta^{13}C_{C4}}$$

$$fC_4 = 1 - fC_3$$

where, $\delta^{13}C_{PLFA}$ is the δ^{13}C value (‰) of the PLFA; $\delta^{13}C_{C3}$ is the δ^{13}C value of ryegrass; $\delta^{13}C_{C4}$ is the δ^{13}C value of maize; fC_3 is the proportion of C that derived from C3-C; fC_4 is the proportion of C that derived from C_4-C.

2.4 GHG incubations of soils from the conventional treatment (plough)

Five cores of surface soil samples (0–10 cm depth × 4 cm diameter) from the *plough* treatment only were collected in late August 2014 from the same slope points described in 2.2, and composited yielding three soil samples per slope position. Five hundred grams of fresh sieved (4 mm) soil was incubated in 1L Kilner jars for 22 days (528 h) under aerobic conditions (i.e. lids removed until gas sampling) at 20 °C with moisture contents equivalent to 80% WFPS to create conditions favourable for denitrification. Sporadic but heavy rainfall events are frequent in the SW of England (Fig. S3), so these soil moisture conditions, representing high anaerobicity within a short period, are typical for topsoils during summer storms in the region. Six soil fertilizer treatments (combinations of slope position and N addition) were investigated: (i) *top*; control soil from the top slope position; (ii) *top + N*; soil from the top slope position with N addition; (iii) *mid*; control soil from the mid slope position; (iv) *mid + N*; soil from the mid slope position with N addition; (v) *bottom*; control soil from the bottom slope position; (vi) *bottom + N*; soil from the bottom position with N addition. Ammonium nitrate (NH_4NO_3, 34.5% N) was dissolved in enough distilled water to achieve the target moisture content (WFPS of 80%) and applied to the soils in the N addition (+N) treatments at a rate equivalent to 160 kg N ha^{-1}, providing 118 mg NO_3^--N kg^{-1} and 118 mg NH_4^+-N kg^{-1}. The same volume of distilled water was applied to the soils with no N addition. Nitrous oxide, CH_4 and CO_2 emissions and soil mineral N (NO_3^--N and NH_4^+-N) were sampled daily from day 0 to 9, and then on days 12, 15 and 21.

Greenhouse gases (CO_2, CH_4 and N_2O) were collected from the incubated soil samples and analysed according to Garcia-Marco et al. (2014) to give the potential for denitrification of these soils. At the time of sampling, the Kilner jars were sealed in sequence so that time-zero samples could be taken through a rubber septum in the lid. Gas samples were collected from the headspace with a syringe and transferred to pre-evacuated 22 ml gas vials for analysis. The gas samples were collected again after 20, 40 and 60 min (removing a total of 10% of the Kilner jar volume). At the end of each gas sampling session, the jars were weighed, and moisture content adjusted if necessary. All gas samples were analyzed using a Perkin Elmer Clarus 580 GC with a Turbo Matrix 110 headspace auto-sampler filled with a 63 Ni Electron Capture Detector (for N_2O) and an FID fitted with a methanizer for CO_2 and CH_4 analysis with two identical Elite Plot Q mega-bore capillary columns (Perkin Elmer, Shelton,

Connecticut, USA). The GC system had a minimum detectable amount of 0.167, 0.190 and 0.012 ppm for CO_2, CH_4 and N_2O, respectively. Samples were analyzed within two days of collection.

A parallel incubation for destructive soil sampling was set up under the same conditions as the Kilner jar incubation for gas sampling. Twenty-one replicate glass tubes were filled with 25 g soil at the same depth to the soils in the Kilner jars for each treatment giving a total of 126 soil samples. These were treated the same as the Kilner jar samples, so water and N were added at the same proportion to provide soil moisture that equivalent to 80% WFPS and N rate of 160 kg N ha^{-1}. Three replicate tubes of each of the six treatments were removed for mineral N immediately after each gas sampling event.

2.5 Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 22.0 (IBM Inc., USA). Data were calculated as arithmetic means with standard errors. Duncan's test was used for comparison between pairs of means at the 5% level. A mixed effects analysis of variance (ANOVA) approach for split-plot design was used to analyze the significance of differences between pH, SOC, TN, mineral N contents, $\delta^{13}C$ and $\delta^{15}N$ values of bulk soils, and the concentration and $\delta^{13}C$ value of extracted PLFAs, using slope position and tillage treatment as fixed factors and block as random effect. For multiple measured variables of the incubation experiment, a repeated measures approach of ANOVA was first applied to address the temporal variations during the incubation. Then at each measured time, the significance of differences between values for fluxes and cumulative emissions of greenhouse gases (N_2O, CO_2 and CH_4), and mineral N contents from incubated soils sampled from different slope positions was tested with Two-way ANOVA. The correlations between emissions of N_2O, CO_2, CH_4, and chemical characteristics of incubated soils were presented by Spearman correlation coefficient (r).

3 Results

3.1 Soil properties

3.1.1 Differences between soil properties at different slope positions

There was no significant difference between the soil water content (range 9.3–12.6%) of any slope point or between treatments in July 2014 (Table 1). Significant differences between slope position and management (but not slope position × management) were determined for soil pH, %SOC and %TN. Soil pH values ranged from neutral to slightly acidic, and decreased significantly downslope, ranging from 6.7 to 7.2 at the top slope position for *plough* and min-*till*, respectively and from 6.2 to 6.5 at the slope foot for min-*till* and *plough,* respectively (Table 1). %SOC was low and ranged from 1.2% (*plough,* foot position) to 1.7% (*strip-till*, top position). %SOC at the top slope position was significantly larger than the lower slope positions in *plough* (bottom and foot positions) and min-*till* (foot position), and was significantly greater in any slope position under *strip-till* (Table 1). %TN ranged from 0.12% (*plough* and min-*till*; foot position*)* to 0.21% (*strip-till*, top and bottom position). Soil C:N ratios were low (~7:1) and similar for all samples and showed no difference between management or slope position. Slope position significantly affected NO_3^--N and NH_4^+-N concentrations regardless of tillage treatment. NO_3^--N concentrations at the bottom and foot slope positions were ~ three (*plough* and min-*till*) to ~ 11 times greater (*strip-till*) than the top and mid slope positions, and always largest at the bottom slope position (Table 1). The bulk soil stable $\delta^{13}C$ and $\delta^{15}N$ values were not significantly different at any slope position under any management.

Table 1

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Characteristics of surface soil samples (0–10 cm) collected from four slope positions (Top, Mid, Bottom, Foot) under three maize management treatments (*plough*, min-*till* and *strip-till*) in late July 2014. Data are presented as mean values with standard errors in brackets (n = 3). Values within the same column of each management followed by different lowercase letters indicate significant differences between slope positions at $p < 0.05$ level. Different uppercase letters indicate significant differences between managements. Results of the split-plot approach of ANOVA are presented as F value followed by test of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *ns*, $p > 0.05$). Highlighted in bold are those significant factors.

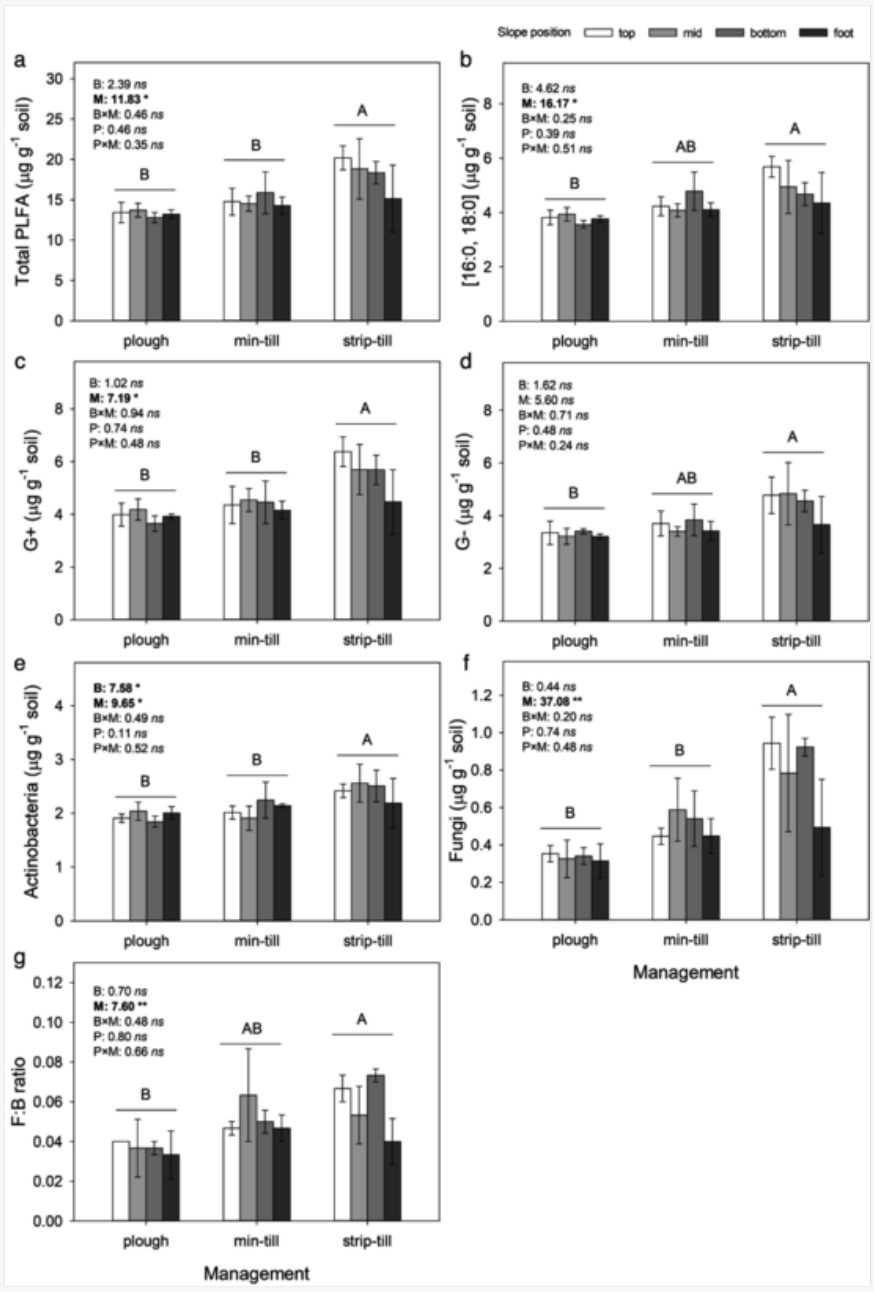
Management	Slope position	pH	SOC (% DM)	TN (% DM)	C:N ratio	NO_3^--N (mg N kg^{-1} DM)	NH_4^+-N (mg N kg^{-1} DM)	Soil water content (%)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Plough	Top	7.15 (0.12) a	1.39 (0.02) a	0.19 (0.002) a	7.28 (0.12) a	20.6 (3.6) b	0.20 (0.09) b	12.6 (0.3) a	−26.0 (0.28) a	6.5 (0.13) a
	Mid	6.96 (0.15) a	1.33 (0.04) ab	0.18 (0.005) a	7.40 (0.25) a	31.3 (10.7) b	0.06 (0.03) b	11.8 (1.0) a	−26.0 (0.08) a	6.4 (0.25) a
	Bottom	6.96 (0.04) a	1.26 (0.01) bc	0.18 (0.003) a	6.95 (0.21) a	87.2 (15.3) a	0.26 (0.12) b	10.6 (1.1) a	−25.5 (0.53) a	6.2 (0.04) a
	Foot	6.53 (0.09) b	1.21 (0.03) c	0.17 (0.003) b	7.20 (0.34) a	69.4 (12.4) a	4.36 (1.63) a	9.3 (1.5) a	−26.1 (0.38) a	6.2 (0.21) a
		A	B	B	A	A	A	A	AB	A
Min-till	Top	6.67 (0.06) a	1.43 (0.05) a	0.19 (0.008) a	7.49 (0.12) a	26.2 (4.3) b	0.24 (0.11) b	11.1 (0.6) a	−25.0 (0.33) a	6.3 (0.09) a
	Mid	6.59 (0.06) a	1.36 (0.01) ab	0.18 (0.001) a	7.41 (0.15) a	31.3 (2.9) b	0.23 (0.13) b	9.7 (0.6) a	−26.0 (0.40) a	6.1 (0.27) a
	Bottom	6.41 (0.12) ab	1.35 (0.05) ab	0.18 (0.001) a	7.35 (0.18) a	99.8 (18.1) a	2.71 (1.59) ab	10.1 (0.8) a	−25.8 (0.41) a	6.0 (0.14) a
	Foot	6.15 (0.15) b	1.24 (0.05) b	0.17 (0.006) a	7.41 (0.16) a	88.1 (24.4) a	6.09 (2.34) a	9.6 (0.8) a	−25.6 (0.11) a	6.0 (0.15) a
		B	B	B	A	A	A	A	A	A
Strip-till	Top	6.89 (0.17) a	1.67 (0.05) a	0.21 (0.005) a	7.58 (0.24) a	8.4 (2.5) b	0.74 (0.37) b	10.3 (0.4) a	−26.3 (0.19) a	6.5 (0.36) a
	Mid	6.52 (0.18) ab	1.46 (0.12) a	0.20 (0.009) a	7.15 (0.23) a	15.1 (5.8) b	1.56 (0.46) ab	10.3 (0.6) a	−26.2 (0.36) a	5.9 (0.24) a
	Bottom	6.35 (0.10) b	1.49 (0.07) a	0.21 (0.006) a	7.46 (0.28) a	97.0 (9.1) a	4.61 (1.65) a	10.6 (0.5) a	−26.1 (0.08) a	6.3 (0.29) a
	Foot	6.21 (0.02) b	1.41 (0.10) a	0.20 (0.008) a	7.38 (0.05) a	86.8 (21.9) a	4.41 (0.12) a	11.6 (0.3) a	−25.9 (0.13) a	5.9 (0.21) a
		B	A	A	A	A	A	A	B	A
Two-way ANOVA										
Block (B)		1.02 ns	**12.06 ***	5.25 ns	2.66 ns	**12.83 ***	0.66 ns	4.48 ns	4.26 ns	0.37 ns
Management (M)		**13.46 ***	**116.26 *****	**61.00 ****	1.56 ns	2.38 ns	1.43 ns	1.72 ns	**8.49 ***	3.01 ns

B × M	1.49 ns	0.10 ns	0.34 ns	0.86 ns	0.34 ns	1.98 ns	1.55 ns	0.33 ns	0.42 ns
Slope position (P)	**15.90** ***	**5.19** **	**4.52** *	0.26 ns	**30.45** ***	**13.99** ***	2.94 ns	0.39 ns	1.67 ns
P × M	0.69 ns	0.32 ns	0.37 ns	0.94 ns	0.47 ns	1.31 ns	2.32 ns	1.17 ns	1.46 ns

3.1.2 Effects of management and slope position on PLFA

The concentration of PLFA biomarkers for microbial groups was significantly affected by management, but there was no significant difference between different slope positions under any management (Fig. 1). The concentrations of biomarker PLFAs extracted from *strip-till* soils generally increased compared to those from min-*till* and *plough* in any slope position (Fig. 1). Biomarker PLFAs for Gram positive bacteria (range 3.6 ± 0.3 μg g^{-1}, *plough*, bottom position, to 6.4 ± 0.6 μg g^{-1}, *strip-till*, top position; Fig. 1c) and Gram negative bacteria (range 3.2 ± 0.1 μg g^{-1}, *plough*, foot position, to 4.8 ± 1.2 μg g^{-1}, *strip-till*, mid position; Fig. 1d) were significantly more abundant than Actinobacteria (range 1.8 ± 0.1 μg g^{-1}, *plough*, bottom position, to 2.6 ± 0.4 μg g^{-1}, *strip-till*, mid position; Fig. 1e) and fungi (range 0.3 ± 0.1 μg g^{-1}, *plough*, foot position to 0.9 ± 0.1 μg g^{-1}, *strip-till*, top position; Fig. 1f). Fungal-to-bacterial ratios (F:B) were significantly lower in *plough* (0.037 ± 0.004) compared to that in *strip-till* (0.058 ± 0.006), but were not significantly different between different slope positions (Fig. 1g).

Fig. 1



Summed concentrations of total PLFA and biomarker PLFA for Gram positive bacteria (G +), Gram negative bacteria (G-), Actinobacteria, Fungi and non-biomarker [16:0,18:0] extracted from soils collected from four slope positions under *plough*, min-*till* and *strip-till* management. Error bars represent standard error (n = 3). Results of the split-plot approach of ANOVA are presented as F value of block (B), management (M), slope position (P) and their interactions (B × M, M × P), followed by test of significance (* $p < 0.05$; ** $p < 0.01$; *ns*, $p > 0.05$). Different uppercase letters above the bars indicate significantly differences between managements.

There were differences in the $\delta^{13}C$ values of extracted PLFAs caused by both slope position and management (Table 2; Fig. 2). The $\delta^{13}C$ values of biomarker PLFA for Gram positive bacteria, Gram negative bacteria and fungi were relatively enriched under *plough* (Fig. 2a) and min-*till* (Fig. 2b). The proportions of maize-C incorporated by Gram positive bacteria were similar between four slope positions under *plough* (range 42.1 ± 0.2% to 43.6 ± 1.3%; Fig. 3a) and min-*till* (range 41.2 ± 1.8% to 45.8 ± 3.0%; Fig. 3b). The proportion of maize-C incorporated by Gram negative bacteria and fungi tended to decrease downslope under *plough* management (35.2 ± 2.7% at the top position vs. 26.3 ± 2.4% at the slope foot, 48.5 ± 1.8% at the top position vs. 37.4 ± 5.4% at the slope foot, respectively). Actinobacteria incorporated relatively less maize-C than other microbial groups under *plough* and min-*till* (27.5% and 15.7% at most, respectively) and tended to increase maize-C incorporation downslope with largest values at the slope foot. Under *strip-till* the $\delta^{13}C$ values of biomarker PLFA for all microbial groups (except Actinobacteria) were significantly lower (Fig. 2c) compared to that under *plough* and min-*till*, which corresponded to the decreased proportion of maize-C that was incorporated by microbes (maximum 34.1%; Fig. 3c). Nevertheless, under *strip-till* treatment the $\delta^{13}C$ values of biomarker PLFA for all microbial groups were markedly increased at the foot slope position (range −26.0 ± 0.2‰, Actinobacteria, to −23.5 ± 0.5‰, Gram positive bacteria) compared to the top slope positions (range −31.2 ± 0.2‰, Actinobacteria, to −26.8 ± 0.5‰, Gram positive bacteria), which corresponds to increased maize-C incorporation by these microbial groups downslope (Fig. 3c).

Table 2

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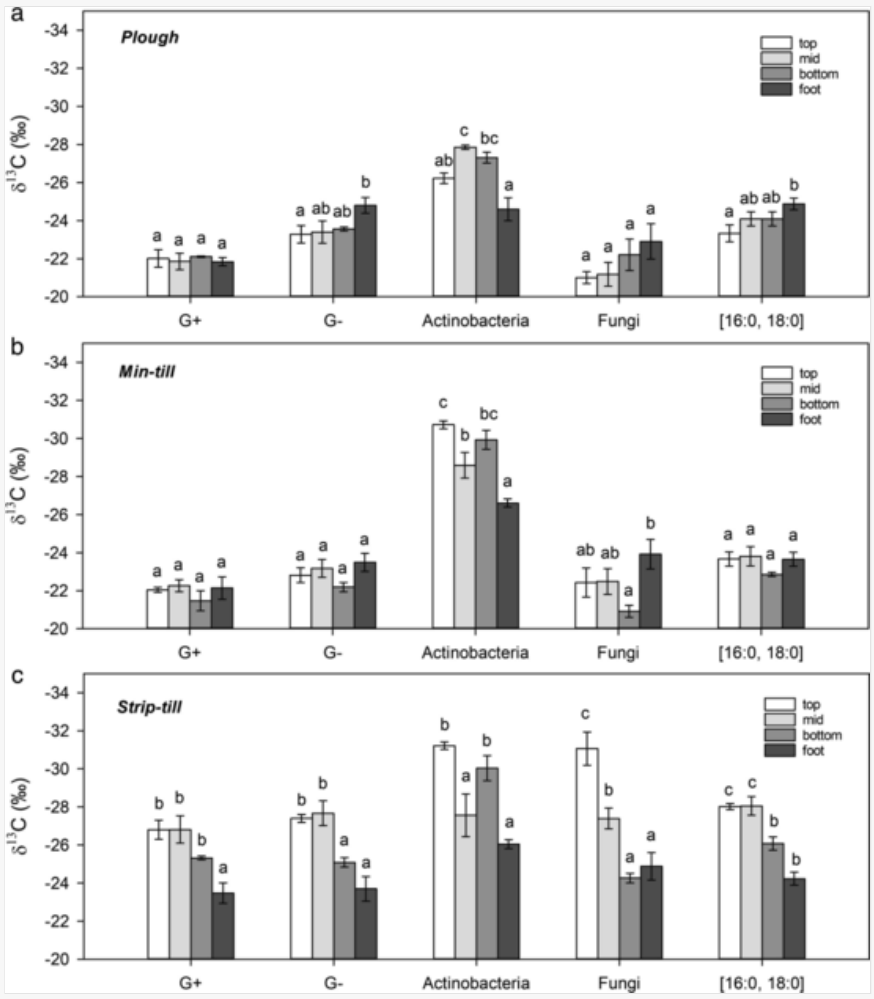
Results of the split-plot approach of ANOVA testing the effects of block, slope position (top, mid, bottom, foot), management (*plough*, min-*till* and *strip-till*), and their interactions on the (a) $\delta^{13}C$ values of biomarker PLFAs, and (b) proportion of maize-C (C_4-C) that incorporated into PLFAs for different microbial groups. Results of ANOVA are presented as F value with test of significance (p value) in brackets. Highlighted in bold are those significant factors.

	Gram positive bacteria	Gram negative bacteria	Actinobacteria	Fungi	[16:0, 18:0]
(a) *PLFA* $\delta^{13}C$ *value*					
Block (B)	0.84 (0.494)	1.28 (0.372)	0.06 (0.943)	4.72 (0.089)	3.72 (0.122)

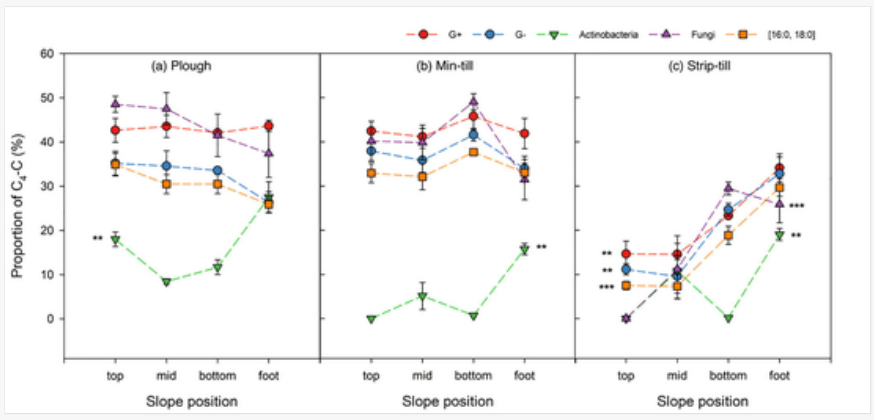
Management (M)	**78.54 (0.001)**	**40.35 (0.002)**	**21.53 (0.007)**	**207.19 (<0.001)**	**178.63 (<0.001)**
B × M	1.24 (0.328)	1.43 (0.265)	1.28 (0.315)	0.30 (0.873)	0.43 (0.785)
Slope position (P)	**5.20 (0.009)**	**4.52 (0.016)**	**30.40 (<0.001)**	**5.81 (0.006)**	**5.97 (0.006)**
P × M	**4.75 (0.005)**	**10.68 (<0.001)**	**4.89 (0.004)**	**9.15 (<0.001)**	**11.12 (<0.001)**
(b) *Proportion from C_4-C*					
Block (B)	0.84 (0.494)	1.28 (0.372)	0.92 (0.470)	**12.01 (0.020)**	3.72 (0.122)
Management (M)	**78.54 (0.001)**	**40.35 (0.002)**	**37.86 (0.003)**	**348.96 (<0.001)**	**178.63 (<0.001)**
B × M	1.24 (0.328)	1.43 (0.265)	0.54 (0.706)	0.18 (0.946)	0.43 (0.785)
Slope position (P)	**5.20 (0.009)**	**4.52 (0.016)**	**25.40 (<0.001)**	**4.49 (0.016)**	**5.97 (0.006)**
P × M	**4.75 (0.005)**	**10.68 (<0.001)**	**2.93 (0.036)**	**7.34 (<0.001)**	**11.12 (<0.001)**

Fig. 2



Mean $\delta^{13}C$ values of biomarker PLFA for Gram positive bacteria (G +), Gram negative bacteria (G-), Actinobacteria, fungi and non-biomarker [16:0,18:0] extracted from soils from top, mid, bottom and foot slope positions under (a) *plough*, (b) min-*till* and (c) *strip-till* management. Error bars represent standard error (n = 3). For each microbial group, different letters above the bars indicate significant differences at $p < 0.05$ level.

Fig. 3

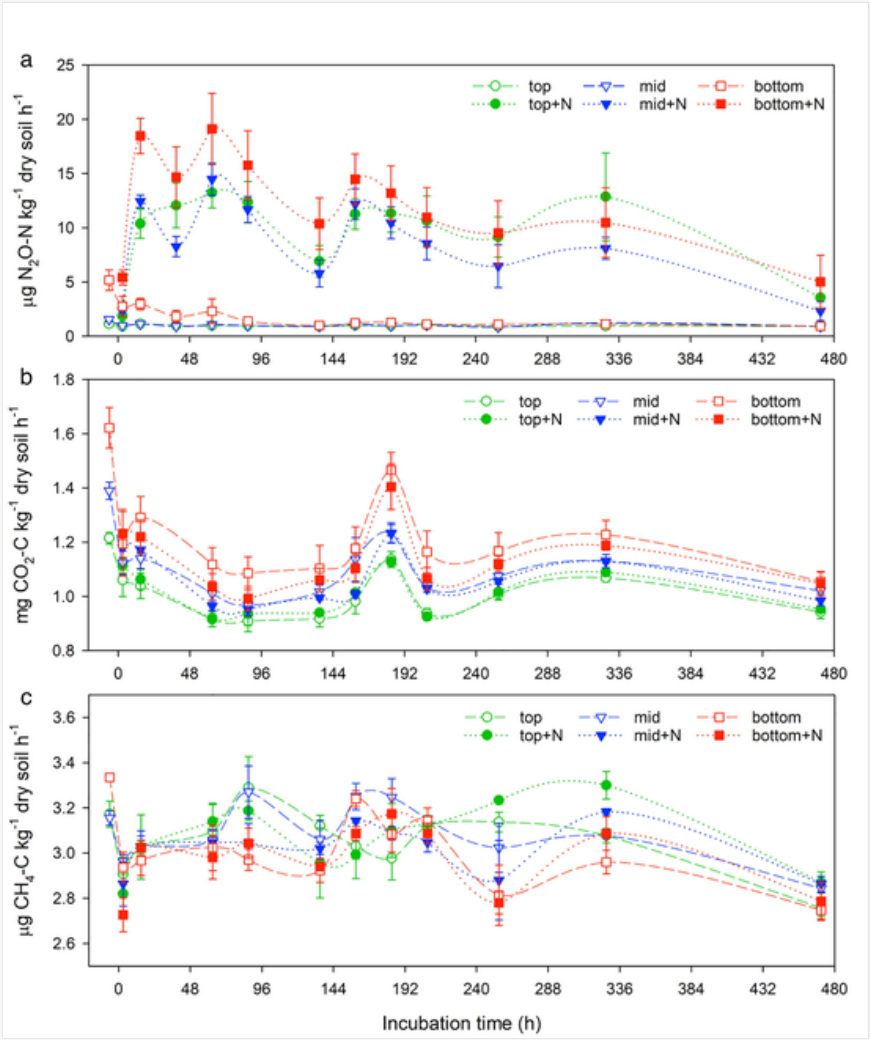


Percentage of maize-C (C_4-C) incorporated into biomarker PLFA for Gram positive bacteria (G +), Gram negative bacteria (G-), Actinobacteria, fungi and [16:0,18:0] extracted from soils collected from four slope positions under (a) *plough*, (b) min-*till* and (c) *strip-till* management. Error bars represent standard error (n = 3). Asterisks marked on the left / right side of symbols that representing certain microbial group indicate significant differences between slope positions at $p < 0.05$ level. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.2 Greenhouse gas (N_2O, CO_2 and CH_4) emissions from incubated plough soils

Before N application, the emissions of three GHGs were all significantly greater from the bottom slope position than the other slope positions (Fig. 4). The effects of slope position and N addition on N_2O flux from the conventional ploughed soils were both significantly influenced by the time when fluxes were measured during the incubation (Table 3).

Fig. 4



Dynamic changes in daily fluxes of (a) N_2O, (b) CO_2 and (c) CH_4 from incubated soils sampled from three slope positions in *plough* management with N-fertilizer treatment (+N) or control. Error bars represent standard error (n = 3). Time zero indicates the time point when N-fertilizer was added in corresponding treatments.

Table 3

The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Results of the repeated measures approach of ANOVA testing temporal variations of the effects of slope position (top, mid, bottom), N addition (+N, control) and their interaction on GHG (N_2O, CO_2 and CH_4) fluxes and soil mineral N (NO_3^--N and NH_4^+-N) contents. Results are presented as F value with test of significance (*p* value) in brackets. Highlighted in bold are those significant factors.

Source of variance	GHG flux			Soil mineral N content	
	N_2O	CO_2	CH_4	NO_3^--N	NH_4^+-N
Within-subjects Effects					
Time	**45.94 (<0.001)**	**57.66 (<0.001)**	**13.96 (<0.001)**	**163.66 (<0.001)**	**581.02 (<0.001)**
Time × Slope position	**3.03 (0.025)**	2.05 (0.072)	**2.45 (0.001)**	**5.85 (0.001)**	**6.43 (0.002)**
Time × N addition	**42.63 (<0.001)**	1.29 (0.289)	1.91 (0.050)	**143.99 (<0.001)**	**561.13 (<0.001)**
Time × Slope position × N addition	1.77 (0.149)	0.57 (0.776)	0.66 (0.856)	**5.70 (0.001)**	**6.44 (0.002)**
Between-subjects Effects					
Slope position	1.56 (0.250)	**10.71 (0.002)**	**4.11 (0.044)**	**36.47 (<0.001)**	**9.34 (0.004)**
N addition	**75.28 (<0.001)**	0.39 (0.542)	0.57 (0.464)	**4890.25 (<0.001)**	**1076.79 (<0.001)**
Slope position × N addition	0.78 (0.481)	0.50 (0.617)	1.06 (0.376)	2.66 (0.111)	**8.79 (0.004)**

N application in the incubated soils caused a rapid increase in N_2O emissions starting at 3 h that peaked after 63 h (Fig. 4a). Maximum fluxes of 13.2 ± 1.4, 14.5 ± 1.5 and 19.1 ± 3.3 µg N_2O-N kg^{-1} soil h^{-1} were measured from soils collected from top, mid and bottom positions, respectively during the first 24 h after the application of the treatments (Fig. 4a). The fluxes in the N treatment showed several peaks during the 480 h incubation when they reached background values (Fig. 4a). The emissions of N_2O from the bottom position were significantly greater than the other slope positions during the first 15 h (Fig. 4a, Table 4). The N_2O emissions decreased progressively to 3.6 ± 1.3, 2.3 ± 0.9 and 5.0 ± 2.5 µg N_2O-N kg^{-1} soil h^{-1} for top, mid and bottom slope positions, respectively, by the end of the incubation. N_2O emissions from N-treated soils were always significantly larger after 15 h than the control (no N) soils (Fig. 4a, Table 4). At the end of incubation, cumulative N_2O emissions from N-treated soils from top, mid and bottom positions accounted for 4.6 ± 0.9, 3.7 ± 0.5 and 5.3 ± 1.3 mg N_2O-N kg^{-1} dry soil, respectively (Fig. 5a).

Table 4

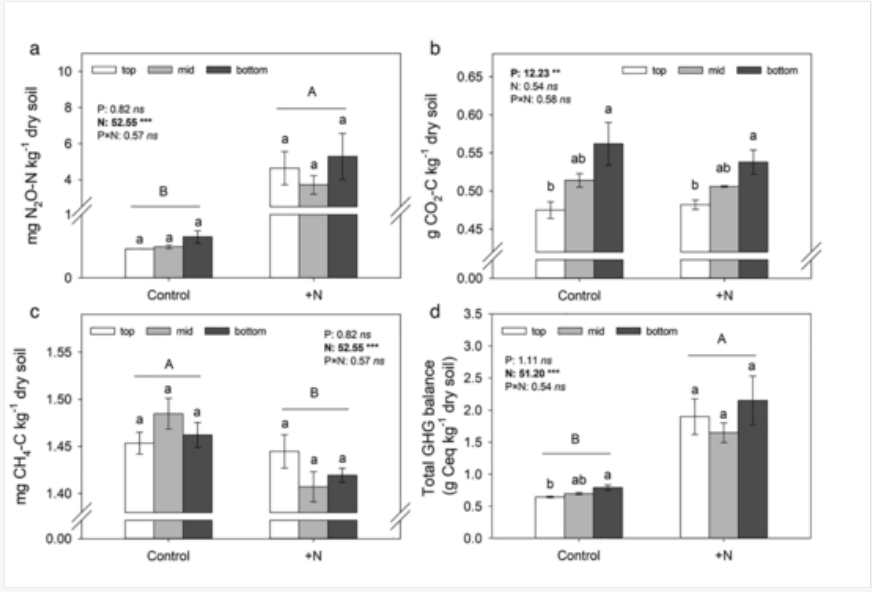
The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Results of two-way ANOVA testing the effects of slope position (P), N-fertilizer treatment (N) and their interaction (P × N) on fluxed of (a) N_2O (b) CO_2 and (c) CH_4 from incubated soils at each sampling time. Results are presented as F value followed by test of significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; *ns*, $p > 0.05$). Highlighted in bold are those significant factors.

GHG flux	Time (hours after addition of N-fertilizer)											
	3	15	39	63	87	135	159	183	207	255	327	471
(a) N_2O												
P	**19.38** ***	**16.31** ***	3.02 *ns*	2.72 *ns*	1.16 *ns*	1.92 *ns*	0.99 *ns*	0.63 *ns*	0.36 *ns*	0.57 *ns*	0.54 *ns*	0.65 *ns*
N	**17.82** ***	**256.22****	**73.36****	**110.98****	**86.94****	**44.16****	**127.09****	**86.70****	**48.37****	**30.59****	**28.63****	**7.72***
P × N	1.73 *ns*	**5.99***	1.77 *ns*	1.02 *ns*	0.75 *ns*	1.77 *ns*	0.74 *ns*	0.39 *ns*	0.33 *ns*	0.46 *ns*	0.69 *ns*	0.63 *ns*

(b) CO_2												
P	1.63 *ns*	**8.50****	—	**8.66****	**4.42***	**6.49***	3.41 *ns*	**19.18*****	**12.06****	**7.61****	**12.35****	**7.52****
N	0.74 *ns*	0.01 *ns*	—	1.75 *ns*	0.73 *ns*	0.17 *ns*	1.60 *ns*	0.36 *ns*	1.52 *ns*	0.46 *ns*	0.11 *ns*	0.25 *ns*
P × N	0.01 *ns*	0.67 *ns*	—	0.65 *ns*	1.15 *ns*	0.29 *ns*	1.13 *ns*	0.22 *ns*	1.00 *ns*	0.33 *ns*	0.70 *ns*	0.39 *ns*
(c) CH_4												
P	0.61 *ns*	0.13 *ns*	—	0.91 *ns*	3.42 *ns*	1.24 *ns*	**5.78***	1.04 *ns*	0.24 *ns*	**7.02***	**6.38***	1.64 *ns*
N	4.71 *ns*	0.08 *ns*	—	0.002 *ns*	1.36 *ns*	0.94 *ns*	4.36 *ns*	0.06 *ns*	2.50 *ns*	0.10 *ns*	**15.82****	2.18 *ns*
P × N	0.41 *ns*	0.10 *ns*	—	0.13 *ns*	1.41 *ns*	0.68 *ns*	0.51 *ns*	1.43 *ns*	1.08 *ns*	0.66 *ns*	0.89 *ns*	0.53 *ns*

Fig. 5



Cumulative emissions of (a) N_2O, (b) CO_2 and (c) CH_4 from incubated soils sampled from three slope positions in *plough* management with N-fertilizer addition (+N) or control. Error bars represent standard error (n = 3). Results of two-way ANOVA are presented as F values of slope position (P), nitrogen addition (N) and their interaction (P × N), followed by test of significance (** $p < 0.01$; *** $p < 0.001$; *ns*, $p > 0.05$). Different letters above the bars indicate significant differences between slope positions at $p < 0.05$ level with N-fertilizer addition (+N) or control.

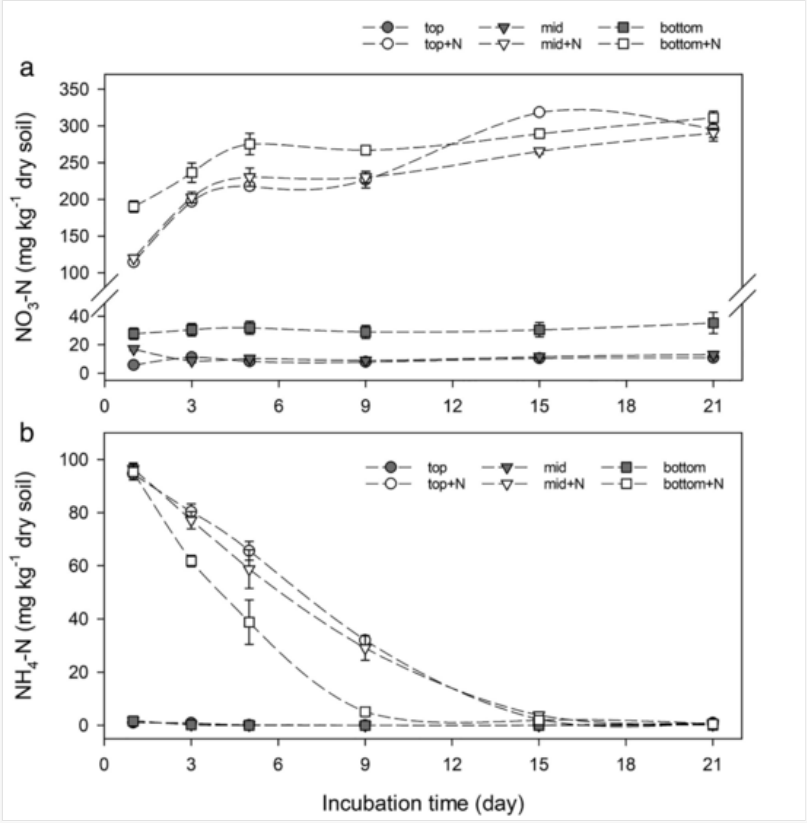
A bimodal CO_2 respiration flux was observed from the incubated soils, with the largest CO_2 fluxes measured at the start of the incubation (1.22 ± 0.02, 1.39 ± 0.03 and 1.62 ± 0.07 mg CO_2-C kg^{-1} soil h^{-1} from the top, mid and bottom slope positions, respectively) and a second peak after 183 h (CO_2 fluxes of 1.13 ± 0.01 to 1.47 ± 0.06 mg CO_2-C kg^{-1} soil h^{-1}, respectively; Fig. 4b). Cumulative CO_2 emissions accounted for 0.48 ± 0.01, 0.51 ± 0.01, and 0.56 ± 0.03 g CO_2-C kg^{-1} dry soil from the top, mid and bottom positions, respectively, by the end of incubation ($p < 0.05$ by Duncan's test; Fig. 5b). The application of N had no significant effect on soil respiration ($p > 0.05$; Table 3). Slope position had significant effects on CO_2 fluxes (Table 4) and cumulative CO_2 emissions: soils from the bottom position had the largest daily CO_2 flux and cumulative emissions during the whole incubation period compared to the top slope position.

The CH_4 emissions from the incubated soils ranged from 2.7 to 3.3 μg CH_4-C kg^{-1} soil h^{-1} during the incubation. The effects of slope position and N addition on CH_4 flux were not consistent at different measuring time, particularly during the first 4 days of incubation (Fig. 4c, Table 4). After 255 h, the CH_4 emissions from the top position (2.9 ± 0.0 to 3.3 ± 0.1 μg CH_4-C kg^{-1} soil h^{-1}, with N addition) were generally larger than that from the bottom position. Cumulative CH_4 emissions accounted for 1.4 to 1.5 mg CH_4-C kg^{-1} soil from all incubated soils, and was significantly lower from soils with N-fertilizer treatment (Fig. 5c). The total GHG balance (N_2O, CH_4 and CO_2) gives 0.65 ± 0.01, 0.70 ± 0.02 and 0.79 ± 0.04 g Ceq kg^{-1} dry soil for the no N treatments (top, mid and bottom, respectively), and 1.9 ± 0.3, 1.6 ± 0.2 and 2.1 ± 0.4 g Ceq kg^{-1} dry soil for the N treatments (top, mid and bottom, respectively) (Fig. 5d). The total GHG emission was significantly higher from incubated soils from the bottom slope position compared to top and mid slope positions ($p < 0.05$).

3.3 Mineral N dynamics in incubated plough soils

Before the incubation started, NO_3^--N concentrations in soils from the bottom slope position (26.4 ± 4.4 mg N kg^{-1} dry soil) were significantly greater than from the top and mid slope positions (5.2 ± 0.4 and 5.4 ± 1.4 mg N kg^{-1}, respectively). NO_3^--N concentrations in the soils from the top, mid and bottom slope positions increased to 217.9 ± 5.2, 230.3 ± 12.4 and 275.3 ± 14.6 mg kg^{-1} five days after N addition, respectively, and 295.5 ± 16.2, 290.2 ± 6.3 and 311.1 ± 9.1 mg kg^{-1} at the end of incubation (Fig. 6a). Soils from the bottom slope position had significantly higher NO_3^--N concentrations than the top and mid slope for the whole incubation period, except for a dramatic increase from top position soils on day 15 (Fig. 6a, Table S1).

Fig. 6



Dynamic changes in (a) NO_3^--N and (b) NH_4^+-N contents extracted from incubated soils from top, mid and bottom slope positions under *plough* management with N-fertilizer treatment (+N) or control. Data are presented as mean values with standard errors in brackets (n = 3).

NH_4^+-N concentrations were not significantly affected by slope position before the incubation started (0.36 ± 0.09, 0.28 ± 0.22 and 0.44 ± 0.04 mg kg^{-1} from the top, mid and bottom slope position, respectively). The NH_4^+-N concentration in the soils from three slope positions after N application increased to 94.6 ± 1.1, 96.5 ± 2.2 and 95.3 ± 3.0 mg kg^{-1}, respectively, on day 1, decreasing to 32.0 ± 1.8, 29.0 ± 4.6 and 5.1 ± 0.6 mg kg^{-1}, respectively, on day 9, and 0.6 ± 0.3, 0.3 ± 0.1 and 0.5 ± 0.1 mg kg^{-1}, respectively, at the end of incubation (Fig. 6b). The depletion of NH_4^+-N in bottom position soils was significantly faster than soils from the other two slope positions between day 1 to day 9 after N application (Table S1).

3.4 Effects of soil chemical characteristics on N_2O and CO_2 emissions from incubated plough soils

Both daily fluxes and cumulative emissions of N_2O had a strong positive correlation with the initial soil NO_3^--N concentrations, but a negative correlation with SOC content (Table S2). The influence of soil NO_3^--N and SOC contents on N_2O emissions were most significant before 87 h, but became weaker or insignificant by the end of incubation. An effect of soil C:N ratio on cumulative N_2O emissions was expressed at the beginning of the incubation (before 63 h). Similar to N_2O emissions, the initial contents of NO_3^--N and SOC were the main factors affecting both daily fluxes and cumulative emissions of CO_2 from soils from different slope positions (Table S2). The daily fluxes of CH_4 were not significantly correlated with any of initial soil properties, and the cumulative emissions between 159 h and 255 h were positively correlated with initial NH_4^+-N concentration.

The initial fluxes of three GHGs before incubation had a strong positive correlation with the initial soil NO_3^--N concentrations, but a negative correlation with SOC content (Table 5). Similarly, the cumulative emissions of N_2O and CO_2, and total GHG balance (CO_2 equivalence) at the end of incubation were all positively correlated with initial soil NO_3^--N concentration, and negatively correlated with SOC content.

Table 5

ⓘ The table layout displayed in this section is not how it will appear in the final version. The representation below is solely purposed for providing corrections to the table. To preview the actual presentation of the table, please view the Proof.

Spearman correlation coefficients (r) of GHG emissions with initial chemical properties of soils sampled from the top, mid and bottom positions along the slope profiles in plough cultivation. Significant factors are highlighted in bold. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	Initial GHG fluxes before incubation			**Cumulative emissions at the end of incubation**			**Total GHG emission**
	N_2O	**CO_2**	**CH_4**	**N_2O**	**CO_2**	**CH_4**	
pH	−0.283	−0.500	0.067	−0.383	−0.433	−0.100	−0.450
SOC	**−0.728***	**−0.862****	−0.268	**−0.695***	**−0.753***	−0.318	**−0.770***
TN	−0.260	−0.433	0.000	−0.520	−0.606	0.087	−0.606
C:N	0.400	0.433	−0.300	0.450	0.383	0.050	0.417
NO_3^--N	**0.933*****	**0.833****	**0.669***	**0.700***	**0.683***	0.083	**0.667***
NH_4^+-N	0.067	0.059	0.544	−0.218	−0.100	0.452	−0.192

4 Discussion

4.1 Carbon and nitrogen dynamics at different slope positions

The SOC content of the soils at the experimental site was low regardless of treatment (maximum values <2%, and C:N ratio of 7:1) and the differences in %SOC values and C:N ratios between different slope positions were <0.5% and 0.45, respectively. Such low C-content and low C:N soils were reported for similar clay loam soils managed for cereal cropping, e.g. 1.76% and 7:1, respectively in southeast England (Karhu et al., 2014). The top slope positions in this experimental field had significantly larger SOC than the lower slope positions (Table 1), which is in consonance with the results found in a previous study of an eroding arable field after 2 years' maize cultivation in Scotland (Dungait et al., 2013a). This supports the theory of dynamic replacement, which states that C lost from top slope positions by tillage erosion is rapidly replaced by crop-derived C adsorbing to new exposed mineral facies (Harden et al., 1999; Doetterl et al., 2012). Because adsorption to mineral facies strongly protects bound carbon making it biologically unavailable, an increase in bulk SOC content in degraded soils does not automatically indicate its potential activity. Furthermore, there were no significant differences in the bulk $\delta^{13}C$ values of the soils at any slope position under any tillage management, indicating that the min */ strip till* management treatments super-imposed on an existing maize-cropped system did not have strong additional effect on bulk SOC dynamics. This might also indicate the C3 inputs from the strip till management were rapidly lost by erosion from the sloping site or by rapid mineralization in this C-poor soil system. We did not measure litter loss by erosion or decomposition over time indicating the need for further investigations exploring these aspects. It is also possible that the yearly fertilization with urea influenced the C utilization by microbes, but this effect is not able to separate with our experimental design.

Total N content did not differ between slope positions, but the largest NO_3^--N concentrations were observed at the bottom slope position which were similar to that reported previously (Quinton et al., 2010). The experimental site was part of a project involving the site in this study (in Devon) and also in eastern England (in Norfolk). Total nitrate leaching across both sites was significantly greater under conventional management than strip tillage (Norris, 2015) but the ryegrass cover could have had an effect here. There was tendency for NH_4^+ to increase at bottom slope positions that differed between *plough* and min *till* and *strip till* treatments. The source may be eroded sediment-bound NH_4^+ or the ammonification of organic N from organic matter, but we did not explore different sources in this study. The low C:N ratios at all slope positions under all treatments suggests that the soils was severely degraded by years of conventional arable cultivation. Crucially, a C-to-N ratio < 8 can cause N release, and, therefore, an increase in microbial activity or growth is not expected by increasing N availability under C-limited conditions, as the vital energy source is absent (Kuzyakov et al., 2000). Under such circumstances, biologically available pools of C would have been mineralised extremely rapidly. The half-life of soluble organic substrates, including simple sugars and amino acids, is<1 h (Boddy et al., 2007).

4.2 Microbial community dynamics at different slope positions

Phospholipid fatty acid analysis is regularly used to understand the broad community composition of soil microorganisms (Frostegård et al., 2011). Biomarker fatty acids correspond to microbial groups with different morphologies that tend to have different life strategies and ecosystem functions (Waldrop and Firestone, 2004; Wixon and Balser, 2013).

The F:B ratios (0.02 to 0.08) measured in the *plough* soils using PLFA biomarkers were similar to those previously reported for maize cultivation (0.05 to 0.12; Spedding et al., 2004). By comparison, min-*till* and *strip-till* significantly increased the F:B ratio (Fig. 1g) because reduced tillage increases fungal dominance in soil microbial communities (Strickland and Rousk, 2010). Nonetheless, the low SOC across all management systems regardless of tillage treatment suggests that >2 years are required to improve soil quality (soil physical, chemical and biological function) in a rapidly eroding, degraded arable soil. Therefore, caution must be taken when describing the 'benefits' of tillage erosion for C sequestration because the negative effects of soil degradation caused by tillage could outweigh the advantages of comparatively minor increases in SOC observed at the top slope position of similar eroding slope profiles.

In this study, PLFAs indicating Gram positive bacteria were reasonably abundant in soils under all management treatments, and had significantly enriched $\delta^{13}C$ values, especially in *plough* and min-*till* soils (Fig. 2). In this sense, the differences observed in the $\delta^{13}C$ values of extracted PLFAs indicate varying proportions of maize-C that was incorporated into PLFA between different microbial groups (Dungait et al., 2013b). In a previous experiment, Gram positive bacteria incorporated more ^{13}C-glucose but demonstrated the least decline in ^{13}C contents over time (Dungait et al., 2013b), reflecting the reported conservative behaviour of Gram positive bacteria as '*K*' strategists that incorporate C into intracellular reserves and utilize it more slowly and independently of supply (Blagodatskaya et al., 2007). By comparison, the Gram negative bacteria, that were a greater proportion of the microorganisms identified, decreased in concentration under *plough* and min-*till* compared with *strip till* (Fig. 1) and also incorporated less maize-derived C than Gram positive bacteria in all management treatments (Fig. 3). Described broadly as opportunistic '*r*' strategists that respond rapidly to changes in labile substrate availability, their comparatively large abundance and preferential use of C_3-substrates (indicated by the relatively depleted $\delta^{13}C$ values) suggest that a biologically available form of 'older' C was available in the soils under *plough* and min-*till*, such as release from slaking aggregates during water erosion. (The input of C3 carbon from cover crops in the *strip till* treatment did not allow similar source apportionment.) In a longer-term experiment (~30 years) in a sloping field in Ohio, tillage and residue removal both increased the proportion of eroded older (C_3), rather than new (C_4, maize-derived) of SOC collected after a rainfall simulation (Beniston et al., 2015).

Previous research using bulk stable ^{13}C isotope analysis of soil microbial biomass C extracted from top soils sampled from different slope positions indicated that microbes responded differentially to erosion-induced redistribution of SOC extracted after 2 years of conventional maize cropping (Dungait et al., 2013a). Similarly, our study determined that a large proportion of previously stabilized SOC (i.e. C_3-C; older than 12 years in our experiment) was being utilized by soil microorganisms in downslope positions in the *plough* and min-*till* treatments (56.4–73.7%, and 58.1–84.2%, respectively, at the slope foot; Fig. 3). Blagodatskaya et al. (2011) also reported that a substantial proportion (60%) of 'old' C_3-C was being mineralized and lost as CO_2 after a switch from C_3 to C_4 cropping for 12 years, despite an 80% contribution of 'new' C to the soil microbial biomass C, suggesting differential routing of C of different source to catabolic and anabolic pathways by soil microorganisms. In this study, we detected preferential and contrasting utilization of SOC with different origins (maize-C or previously stabilized SOC older than 12 years) by specific microbial groups, perhaps indicating trophic niche differentiation observed for other soil organisms in the resource-poor soil environment (e.g. earthworms; Dungait et al., 2008). For example, in contrast to fungi, Actinobacteria utilized a greater proportion of C_3-C, especially at the top slope position (~100% under min-*till* management; Fig. 3b). This supports previous observations, based on ^{13}C-PLFA analysis after addition of different ^{13}C-labelled substrates, that indicated preferential use of specific substrates by this group of microorganisms in arable soil, but not in permanent grassland soil (Dungait et al., 2013b).

The effect of erosion on availability of available C and N (reactive N) in sloping arable fields is rarely considered together, but is of critical importance for the efficient management of the biogeochemical cycles (nutrients and C) in agricultural systems. Studies of the stoichiometry of natural terrestrial systems often report a stable 10:1 ratio of soil C:N, but this balance is extremely perturbed in agricultural systems (Dungait et al., 2012) (i.e. C:N ratio of 7:1 in this study), including substantial modification by erosional processes in sloping landscapes (Quinton et al., 2010) particularly in long-season and non-competitive crops like maize (Beniston et al., 2015; Vogel et al., 2016). In this study, we observed that the proportion of maize-C incorporated into fungal PLFA decreased from ~ 45% at the top slope to 35% at slope foot under *plough* and min-*till* management. This is similar to the study by Dungait et al. (2013a) who found that soil microbial biomass C derived from maize decreased from 54% at the top slope to 41% at the slope foot position. This indicates rapid turnover of eroded whole and particulate organic matter from maize at the downslope positions for topsoils (also observed by Berhe, 2012 in a natural system). We observed large, and relatively increased concentrations of NO_3^- in the downslope positions in July, a considerable time after N fertilizer application in early May. This was presumably derived from the ammonification and subsequent nitrification of organic nitrogen from rapidly decomposing eroded maize crop residues, which may produce nitrate that can be denitrified (Sakala et al., 2000). However, we did not analyse crop residues so we cannot determine the validity of this proposal in this study.

4.3 The impact of slope position on GHG emissions

The potential for GHG emissions from the *plough* treatment only were investigated in an incubation using moistened soil to optimize the conditions, i.e. WFPS, for N_2O release. We simulated a wet period because there is strong evidence that the depositional slope positions have a greater potential for increased N_2O emissions due to high WFPS together with sufficient NO_3^- content and increased labile C availability (Smith et al., 2003; Snyder et al., 2009; Schaufler et al., 2010; Ball et al., 2012). Furthermore, wet summer conditions are regularly experience in the southwest of England, so saturated topsoils are commonplace. An increased potential for N_2O emissions from soils from the bottom slope position compared to the top and mid slope was revealed by both N_2O flux and cumulative emissions from the incubation

experiment (Figs. 4 and 5) in association with the significantly greater topsoil mineral N (NO_3^--N) content. This is similar to studies that measured field rates; e.g. Vilain et al. (2010) quantified N_2O emissions in an agricultural field in Seine Basin, France, and calculated an annual flux of 4.0 ± 2.2 kg N ha^{-1} yr^{-1} at the slope foot compared to 1.1 ± 0.5 kg N ha^{-1} yr^{-1} in an upper slope position, although there are reports showing the direct opposite in incubation experiments (Vilain et al., 2012) or field measurements (Gu et al., 2011).

The largest potential for CO_2 emissions were from the incubated moistened soil from the bottom slope position of the plough treatment, regardless of the addition of N fertilizer (Fig. 4, Table 4), suggesting that the larger NO_3^--N concentrations at the lower slope positions was a driving factor in C mineralisation. CO_2 emissions did not correlate with %SOC content, but the action of soil sieving and the moistening of previously dried soil will have released previously physically protected C from disrupted aggregates and the release of osmoregulants (LMW organic solutes) accumulated at low water potential, driving (Fierer & Schimel, 2003) the initial flush of CO_2. It is also possible that priming effect due to higher C at the lower positions (e.g. higher dissolved C and particulate OM through leaching) would have caused the increased SOM mineralization. However, the C:N ratios of bulk soil were not significantly different between slope positions, indicating that this potential effect was minor. The second CO_2 peak at 183 h was smaller and could be related to the emergence of spore-forming soil microorganisms, i.e. Actinobacteria and fungi, that adapted to utilize more recalcitrant C (i.e. *K* strategists; Dungait et al., 2013b). Abundances of fungi (and presumably mycelial Actinobacteria) regularly disturbed by cultivation recover later (Börjesson, et al., 2015). Temporal shifts in the strength of the detrital feeding channels of soil invertebrates that control the mineralization of plant nutrients and the formation of soil organic matter corresponded to tillage at the experimental site. The isotopic composition of invertebrate taxa indicates that during cultivation only a small proportion of maize derived carbon was consumed by the microbial community (Norris, 2015). However, we did not measure pools of C with different bioavailability in this study, or analyse the PLFA in the incubated soils, so cannot draw further conclusions about controls on CO_2 release in this study.

The incubation experiment indicates that the cumulative emissions of CO_2 and N_2O from moist arable soil, as well as the total GHG balance, were the largest from soils that were collected from the bottom position of the slope sampled in this study (Fig. 5). We acknowledge that this potential applies only to plant-free soil, and that future studies need to capture the influence of plants on C and N exchanges both above and belowground, particularly the microbial hotspot of the rhizosphere. However, the percentage of bare ground at the experimental site could be >80% (Norris et al., 2018) indicating the risk of bare soils under maize cultivation to both erosion and as a source of GHGs, particularly under wet conditions. Considering the climate change scenarios predicted for Western Europe (IPCC, 2007), the results of this study suggest that there is a larger potential for increased GHG emissions (N_2O and CO_2 emissions) from the soil in the lower slope positions in a arable land, and suggest that active management of depositional positions and nutrient applications should be considered to increase nutrient use efficiency across the slope avoiding losses to mitigate climate change.

5 Conclusions

In this study, we investigated the influence of changes in soil C and N properties with slope position on microbial activity from an experimental field system with contrasting maize cultivation treatments. The bulk ^{13}C signature of soils sampled along the slope profile did not suggest a significant down slope movement of maize-derived SOC. However, the significantly larger SOC content in the top slope field positions indicates that C lost from top slope positions by tillage erosion is rapidly replaced by crop-derived C adsorbing to new exposed mineral facies. Compound-specific stable isotope analysis of PLFA extracted from the soils revealed the preferential utilization of recent (<12 years) maize residues by fungi and Gram positive bacteria, and of previously stabilized SOC by Actinobacteria and Gram negative bacteria with more C_3-C incorporated downslope. This suggested enhanced mineralization of previously stabilized SOC at the depositional (bottom and foot) slope positions, confirmed by coincident increased CO_2 emissions from incubated soils. The N dynamics along the slope transect were similar to C, and a greater amount of soil mineral N was quantified at the lower slope positions. The largest emissions of N_2O (and CO_2) from the incubated soils were from those sampled from the bottom slope position suggesting enhanced denitrification, although there was no significant ^{15}N-enrichment of soil N. In summary, we provide evidence to accept the hypothesis that soil conditions in downslope positions in temperate arable fields potentially promote GHG emissions, because the soil conditions in depositional slope positions are favorable for denitrification. As most maize cultivation is situated in fields with erosion susceptible soils in UK, implementing more effective agricultural practices (e.g. no tillage and cover crops) may reduce GHG emissions because of their positive effect to prevent soil erosion and associated movement of C and N.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2021.115171.

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Highlights

- Soil microbial community structure varied at different slope positions under maize cultivation.
- Greater incorporation of old SOC (C3-C) by soil microorganisms in the bottom position of slope.
- Use of maize-carbon is dominated by Gram positive bacteria and fungi.
- Potential CO_2 and N_2O emissions increased at bottom of ploughed slope.
- Carbon sink potential of bottom slopes may be diminished by GHG emissions.

Appendix A Supplementary data

The following are the Supplementary data to this article:

Multimedia Component 1

Supplementary data 1

Queries and Answers

Q1

Query: Your article is registered as a regular item and is being processed for inclusion in a regular issue of the journal. If this is NOT correct and your article belongs to a Special Issue/Collection please contact e.maruthu@elsevier.com immediately prior to returning your corrections.

Answer: This is a regular article

Q2

Query: The author names have been tagged as given names and surnames (surnames are highlighted in teal color). Please confirm if they have been identified correctly.

Answer: Names are correct