Impacts of zero tillage on soil enzyme activities, microbial characteristics and organic matter functional chemistryin temperate soils

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ABSTRACT

Zero tillage management of agricultural soils haspotential for enhancing soil carbon (C) storage and reducing greenhouse gas emissions. However, the mechanisms which control carbon (C) sequestration in soil in response to zero tillage are not well understood. The aim of this study was to investigate the links between zero tillage practices and the functioning of the soil microbial community with regards to C cycling, testing the hypothesis that zero tillage enhances biological functioning in soil with positive implications for C sequestration. Specifically, we determined microbial respiration rates, enzyme activities, carbon source utilization and the functional chemistry of the soil organic matter in temperate well drained soils that had been zero tilled for seven years against annually tilled soils. Zero tilled soils contained 9% more soil C, 30% higher microbial biomass C than tilled soil and an increased presence of aromatic functional groups indicating greater preservation of recalcitrant C.

Greater CO_2 emission and higher respirational quotients were observed from tilled soils compared to zero tilled soils while microbial biomass was 30% greater in zero tilled soils indicating a more efficient functioning of the microbial community under zero tillage practice. Furthermore, microbial microbial enzyme activities of dehydrogenase, cellulase, xylanase, β-glucosidase, phenol oxidase and peroxidase were higher in zero tilled soils. Considering zero tillage enhanced both microbial functioning and C storage in soil, we suggest that it offers significant promise to improve soil health and support mitigation measures against climate change.

Key words: Carbon sequestration, Microbial biomass carbon, Greenhouse gases, Soil enzymes, Soil organic matter, Soil microbial functional diversity

1. Introduction

Soil carbon (C) sequestration in agricultural soil has been suggested as a strategy to mitigate greenhouse gas emissions and improve soil quality [1]. The potential of soil to sequester C is affected by regional climate, soil biophysical and chemical properties and soil management [2]. Zero tillage practices have been shown to improve or to maintain soil organic matter in soil[3] and may provide an important management tool for climate change mitigation.The mechanisms of enhanced C sequestration under zero tillage practices have been attributed to reduced disturbance, changes in soil aggregation [4] and microbial activities in addition to increased C inputs from crop residues [5]. However, the microbial and physico-chemical mechanisms of soil organic matter stabilization and C sequestration related to changes in soil management are not well understood.

Organic matter in soil occurs as a complex heterogeneous mixture of organic compounds and consists of different fractions, each of which varies in their stability against microbial degradation depending on the chemical structure of the organic compounds and the environmental conditions. The biochemically stable fraction of C is reported to have a turnover rate of many thousands of years, while the labile fraction is characterised by decomposition in response to soil management such as tillage and crop rotation [6]. A third intermediary fraction is stabilised by physico-chemical mechanisms [7] which may also be affected by tillage practices. Recently, Fourier Transformed Infrared spectroscopy (FTIR) has been used to study SOM characteristics in soil as it provide information on functional groups and structural entities [8].Such understanding is important to ascertain how SOM composition controls the amount of C sequestered in agricultural soil andthe sensitivity of different functional groups to microbial decomposition processes under different tillage practices.

The C storage in soil is determined by the balance of organic inputs from plants and soil microbial decomposition processes. Microbial decomposition involves conversion of soil organic matter, during which plant and microbial biomass may be converted to more stable organic molecules or be respired and released to the atmosphere as CO_2 or CH_4[9]. Microbial re-synthesis of decaying plant and microbial compounds aid C sequestration and may result in formation of stable organic matter compounds which are resistant to decomposition[10]. However, the extent to which carbon is added to soil from microbial biomass is not known. Due to the continuous addition of substrates from crop residue under zero tillage practices, the pattern of microbial community structure may be distinctly different from the tilled soil [11]. For example, changes in microbial community with respect to increased arbuscular mycorrhizal fungi and shifts in phospholipid fatty acid (PLFA) profiles in response to zero tillage have been reported by Helgason and co-authors [12].

Shifts in the microbial community composition have important implications for soil functioning since different microbial groups produce different soil enzymes which are involved in the dynamics of C in soil [13]. For example, β-glucosidase, cellulase and xylanase are important for decomposition of the labile fraction of plant tissue [14, 15] whereas oxido-reductive enzymes such as phenol oxidase and peroxidase contribute to lignin degradation, humification and soil organic matter mineralisation [16]. Tilled soils have been reported to contain lower enzymatic activity than zero tilled soils [17] in response to shifts in availability of organic substrates[18], in soil moisture, soil temperature, soil aeration and constitution of soil flora and fauna [19] which may have important implications for both greenhouse gas production and soil C storage.

The aim of this study was to test the hypothesis that zero tillage enhances biological functioning in soil with positive implications for C sequestration. Specifically, we expected the microbial community in zero tilled soils to exhibit lower metabolic respiration quotients, and greater enzyme activities. For this we (i) characterized the microbial community functional diversity, microbial respiration and enzyme activities and (ii) soil C content and the functional characteristics of the SOM using FTIR in zero tilled and tilled soils.

2. Materials and methods

2.1 Experimental design and sampling strategy

Soil sampling was carried out from six pairs of intensely tilled farms and zero tilled farms in Leicestershire and Lincolnshire in the East Midlands of UK. Each pair was located directly adjacent to each other and the distances between paired fields never exceeded 10m. The zero tilled soils had been managed in this way for seven years. Selected site characteristics are

presented in Table 1 (see also [3] for more details). In fields under zero tillage, stubble was left at the surface after harvest of the previous crop. Weeds were removed by spraying glyphosate before drilling. Seed drilling was carried out between the root stocks of previous crop using min-till seed drills. The previous crops were either wheat or oilseed rape. Tilled soil sites were annually ploughed to depths of 20-25 cm and contained the same crops as the zero tilled fields.

From each location, five bulk soil samples were collected at random, using a spade from two depths (0 to 10 cm and 10-20 cm referred to as surface and sub-surface respectively), after harvest of the previous crop. Sampling was carried out during October 2012, before any cultivation, and about 1000g of field moist soils were collected in polythene bags. The pooled subsamples were used for analysis. Samples for the study of microbial community structure and soil enzymes were frozen at -20^{o}C and thawed at 4^{o}C over 5 days prior to analysis [20]. One set of samples were retained at 4^{o}C to study greenhouse gas (GHG) flux and microbial biomass C. One set of samples were air dried and passed through a 2 mm sieve. These samples were then oven dried and subjected to ball milling using a planetary ball mill (Retsch, PM400) using agate mortar with the help of four balls, at a speed of 300 rpm for 4 minutes and utilized for total C and N estimation. Particle size analysis was performed following hydrometer method [21] and soil textural classification was made as per European classification [22]. Gravimetric soil moisture content was estimated by oven drying field moist samples at hot air oven at 105^{o}C.

2.2 Soil chemical properties

2.2.1 Total carbon and nitrogen

Total C and N content were determined by dry combustion of ball milled soil samples, using a CN analyser (Flash 112 series, CE instruments) set at a furnace temperature of 900^{o}C, carrier

gas flow of 140 ml min^{-1} and oxygen flow of 250 ml min^{-1}. A soil with known C and N concentration was used as a standard.

2.2.2 Fourier Transform Infrared (FTIR) spectroscopy

FTIR absorption spectra were obtained with a Bruker Tensor 27 FTIR equipped with N_2 purge gas generator and a mercury cadmium telluride (MCT detector), and fitted with an attenuated total reflectance (ATR) module. Initially, and after every 8 samples, a background spectrum was created. Oven dried, ball milled soil samples were placed on the ATR crystal, the arm was then rotated over and turned down to press the sample on to the crystal face. The average of a total of 128 scans was collected for each soil sample. The spectral range collected spanned 400 to 4000 cm^{-1} at a resolution of 1 cm^{-1}. All spectra were normalised before analysis in order to allow direct inter-comparison. When interpreting FTIR spectra, the wavenumber position (x-axis) corresponds to the absorbance bands of particular bond types with specific functional groups, and as such can be identified and assigned readily.

2.2.3 Greenhouse gas flux (GHG) from soil

Prior to the measurements of GHG production, field moist soil samples were equilibrated to 15^{o}C for 24 h. Soil samples of 30 g were placed inside a glass jar of 250 ml volume and fitted with rubber septa in the lid to enable gas sampling. The soil was loosely packed without any bulk density adjustment. Initially ambient air, of equivalent volume to that later removed by sampling, was injected into the headspace once the soil cores were placed inside jars. Gas sampling was performedafter ensuring adequate mixing of the airandundertaken at time intervals of 0, 15, 30 and 60 min after closing the headspace. The collected gas samples were stored in pre-evacuated airtight 12 ml glass vials. Samples were analysed for CO_2, CH_4 and N_2O using gas chromatography. CO_2 was detected using a thermal conductivity detector (TCD), CH_4 using a flame ionization detector (FID) and N_2O using an electron capture detector (ECD)

(GC-2014, Shimadzu). Nitrogen was used as the carrier gas. Gas production rates were calculated using linear regression of the gas concentration against sample time. The GHG data was converted to mass per volume and weight basis by the use of ideal gas equation and the molecular mass of each gas [23].

$$n = \frac{PV}{RT} \quad (1)$$

Where n is the number of moles of CO_2, N_2O or CH_4, P is atmospheric pressure (≈1 atm), V is the volume of head space (dm^{-3}), R is the ideal gas constant (0.08205746 L atm K^{-1} mol^{-1}) and T is the temperature of sampling (273.15 + room temperature in ^oC).

$$E = \frac{nm}{at} \times 1000 \quad (2)$$

Where E= flux of each gas in ng m^{-2} g^{-1} h^{-1}, n = number of moles of CO_2, N_2O or CH_4, m = molar weight of CO_2 (44.01), N_2O (44.01) or CH_4 (16.04), a = area of the soil core in cm^2 and t = time in hours.

Respiration quotients were calculated as CO_2-C production per microbial biomass production per gram of soil per hour as in Basiliko *et al.* [24].

2.3 Soil biological properties

2.3.1 Microbial biomass carbon and nitrogen

Microbial biomass C was estimated using the chloroform fumigation - extraction method of Vance *et al.*[25]. Field moist samples were incubated in the chloroform environment in the presence of soda lime. The extraction was carried out using 0.5 M K_2SO_4 at the start of fumigation in un-fumigated samples and 24 hour after fumigation in fumigated samples. Microbial biomass carbon and nitrogen in the extracts were analysed using a Shimadzu CN

analyser (TOC-V CPH Shimadzu). The results were corrected using the value of 0.45 for both carbon and nitrogen as suggested by Jenkinson and co-authors [26].

2.3.2 Soil microbial functional diversity

Soil microbial carbon utilisation was studied using Biolog GN2 microplates (Biolog Inc., California, USA, supplied by Techno-path Distribution Ltd, Limerick, Ireland). The plates consisted of 95 different C substrates in wells along with a control well without any substrate. The colourlessredox dye (tetrazolium violet), present in each well, is reduced following substrate utilisation and turns purple. The intensity of colour was measured with a plate reader with a filter. Initially, the soils stored at -20^{o}C were thawed over 48h. One gram dry weight equivalent of soil was suspended in 100 ml of ¼ Ringer's solution (Composition of full strength Ringer's solution: 2.25 g NaCl, 0.105 g KCl, 0.12 g $CaCl_2$ and 0.05 g $NaHCO_3$ dissolved in 1 litre of distilled water) to get a soil dilution of 10^2. The suspension was thoroughly mixed before transferring 120 µL of suspension to each well of biolog plates using a multichannel dispensing pipette. The biolog plates were then incubated at 20^{o}C for 5 days. The absorbance of each well in the plates was measured at 595 nm using a microplate reader (BioTek ELX 808, BioTek Instruments, Vermont, USA) initially within 2 h of inoculation and then at 24h intervals for 5 days. The Average Well Colour Development (AWCD) was computed after correcting the readings for the control well and the initial reading. The average colour development for each functional guild was also computed [27].

2.3.3 Soil enzymatic activities

2.3.3.1 Dehydrogenase

To determine dehydrogenase, 5 g of field moist soil was incubated with 1% solution of 2,3,5-triphenyltetrazolium chloride at 25^{o}C for 16h. The triphenylformazan (TPF) was extracted with 25 mL of acetone by shaking vigorously for 2h in the dark. The solution was filtered in a semi

dark room and the intensity of TPF was measured at 546 nm against the known standards and expressed as µg TPF g^{-1} h^{-1} [28].

2.3.3.2 Cellulase

For cellulose activity assessment, field moist soil (10 g) was incubated in 15 ml acetate buffer (2M, pH 5.5) using carboxy methyl as a substrate (15 mL,0.7% w/v) for 24 h at 50°C in a sealed Erlenmeyer flask. Similarly, a control was also prepared using acetate buffer alone. After incubation, 15 mL of substrate solution was added to the controls, and the control and samples were filtered immediately. Reducing sugars released during the incubation period were made to react with potassium hexacyanoferrate (III) in an alkaline medium. The reduced potassium hexacyanoferrate (II) was then allowed to react with ferric ammonium sulphate in an acid medium to form a coloured complex of ferric hexacyanoferrate (II). The intensity of colour was read at 690 nm using a spectrophotometer. The activity of cellulase was expressed as mg GE (glucose equivalents) g^{-1} day^{-1}[29].

2.3.3.3 Xylanase

Field moist soil (5 g) was incubated in 15 ml acetate buffer (2M, pH 5.5) using xylan as substrate (15 mL, 1.2% w/v) for 24 h at 50°C in a stoppered Erlenmeyer flask. The control was similarly incubated after adding only the acetate buffer, but without xylan. After incubation, 15 mL xylan solution was added to the controls, and the control and samples were filtered immediately. Reducing sugars released during the incubation period were made to react with potassium hexacyanoferrate (III) in an alkaline medium. The reduced potassium hexacyanoferrate (II) was then allowed to react with ferric ammonium sulphate in an acid medium to form a coloured complex of ferric hexacyanoferrate (II). The intensity of colour was read at 690 nm using a spectrophotometer. The activity of xylanase was expressed as mg GE (glucose equivalents) g^{-1} day^{-1}[29].

213 *2.3.3.4 β- Glucosidase activity*

214 The measurement of β- Glucosidase activity was based on the method modified from Hoffmann
215 and Dedeken [30]reported by Schinner *et al*. [20]. 5g of field moist samples was incubated with
216 20 mL of acetate buffer (2M) and 10 mL of salicin (35 mM) at 37ºC for 3h. The release of
217 saligenin was determined colorimetrically using 2,6-dibromchinone-4-chlorimide at 578 nm
218 using spectrophotometer. The β- Glucosidase activity was expressed as mg saligenin g^{-1} $3h^{-1}$.

219 *2.3.3.5 Phenol oxidase and peroxidase*

220 The measurement of phenol oxidase and peroxidase was based on Dick [31]. For measurement
221 of phenol oxidase activity, 0.5 g of field moist soil was incubated with 3 mL of acetate buffer
222 and 2 mLof 10 mM L-DOPA (L-3,4-dihydroxy phenylalanine). Incubation was done at 25ºC
223 in a shaking environment (100 rev min^{-1}). This was followed by centrifugation for 10 min at
224 5ºC. The reaction product (dopachrome) was read at 475 nm using a spectrophotometer. The
225 method for peroxidase was same as phenol oxidase, but with an additional step of adding 0.2
226 mL of 0.3% H_2O_2, just before incubation. These enzymes were expressed as µmoldopachrome
227 g^{-1} h^{-1}.

228 *2.4 Statistical analysis*

229 To investigate if contrasting tillage treatments and soil depth influenced soil biological and
230 chemical properties a fully factorial two-way analysis of variance was used including tillage
231 and soil depth as factors and sampling location (Table 1) were included as a block effect in the
232 statistical model. The treatment means were compared at the $P < 0.05$ level using the LSD.

233 For Biologplates, Garland [27]recommended choosing positive values higher than 0.25
234 absorbance could eliminate weak false positive response. Hence the statistical analysis was
235 carried out on mean colour intensity values greater than 0.25. First, a repeated-measures
236 ANOVA using time as a factor and sampling location as a block effect was carried out to assess

237 the effect of incubation time on AWCD and substrate utilization of different functional groups.
238 Second, a two-way analysis of variance was performed to test the effect of tillage and depth on
239 AWCD as well as substrate utilization of different functional groups using sampling location
240 as a block effect. For this, a time point was chosen which had AWCD values between 0.75 and
241 1.0 [27] which was at 120 h of incubation. The substrate-utilization patterns were subjected to
242 principal component analysis (PCA) using standardized data.

243 Multiple linear regressions were used to predict the best model describing the carbon content
244 in soil. The maximal model consisted of all the chemical and biological properties studied in
245 this experiment. By using a stepwise backwards elimination process, only the variables that
246 contributed significantly to the model and reduced the residual sum of squares were retained.
247 For illustrative purposes, we also carried out the single linear regression between the
248 parameters that contributed to the multiple regression models. The statistical software package
249 Genstat (14th Edition, VSN International Ltd, Hemel Hempstead, U.K.) was used for data
250 analysis.

251 **3. Results**

252 *3.1 Soil chemical properties*

253 *3.1.1 Total carbon and nitrogen*

254 Zero tilled soils contained 9% more total C (average of the 0-10 and 10-20 cm layers) in the
255 upper 20 cm soil layer (1.42%) than tilled soil (1.29%) (Table 2, $F_{1,5} = 71.06$, $P<0.001$). The
256 total C content was higher in the surface (0-10 cm) than the subsurface layer (10-20 cm) ($F_{1,10}$
257 = 13.30, $P<0.01$). In zero tilled soils the surface layer contained 14% moreC than in the
258 subsurface, whereas in tilled soil it was 16%. Total N followed a pattern similar to that of C
259 (Tillage treatment: $F_{1,5} = 10.99$, $P<0.05$, Depth: $F_{1,10} = 6.11$, $P<0.05$).

3.1.2 FTIR

The general patterns of the FTIR spectra in tilled and zero tilled soils were similar regarding the overall mineral and organic composition of the soil. Detailed analysis of the FTIR spectra identified 20 absorbance bands corresponding to organic soil constituents[32][32][32][32][32]. Band position (wave-numbers) and their functional group assignment are provided in Table 3. Statistically significant differences in peak intensity between tillage treatments were obtained at two wave numbers namely 709 cm^{-1}(aromatics) and 711 cm^{-1} (aromatics) with greater absorbance band intensity found in zero tilled soil (Table 3 and Fig. 1). For these two aromatic wave numbers, the absorbance band intensity was greater in subsurface than surface soils.

3.1.3 CO_2, CH_4, N_2O fluxes and respiration quotients

The highest CO_2 flux was from tilled soil (5.7 µg m^{-2} g^{-1} h^{-1}) which was 41%greaterthan from zero tilled soil (3.4 µg m^{-2} g^{-1} h^{-1}) (Table 4, $F_{1,5} = 6.9$, $P < 0.05$). The CO_2 flux was higher from the soil surface than from the sub surface soil in both zero tilled and tilled soil ($F_{1,10} = 14.44$, $P < 0.01$). The emission of CH_4 from zero tilled soils (0.85 ng m^{-2} g^{-1} h^{-1}) was 75% higher than from tilled soils (0.20 ng m^{-2} g^{-1} h^{-1}) (Table 4, $F_{1,5} = 18.99$, $P < 0.01$). The emission from surface soil was 59% greater than from the subsurface soil ($F_{1,5} = 6.26$, $P < 0.05$). The mean N_2O flux was higher from zero tilled soil (0.92 ng m^{-2} g^{-1} h^{-1}), although this difference was not significant (Table 4, $F_{1,5} = 1.49$, $P > 0.05$). Soil depth and its interaction with tillage did not affect the N_2O flux significantly. The respiration potential varied significantly with tillage practice. Tilled soil had a higher respiration quotient than zero tilled soils, with 17.0and 17.1µg CO_2-C per microbial biomass carbon per hour at the surface and subsurface, respectively, which was 35 and 43% higher, respectively, than in the surface and subsurface soil from zero tilled soil (Table 4, $F_{1,5} = 14.15$, $P < 0.05$). The respiration quotient increased with depth in both zero tilled and tilled soils, however this effect was not significant.

3.2 *Soil biological properties*

3.2.1 *Microbial biomass carbon and nitrogen*

Zero tillage increased microbial biomass C in soil by30% when averaged across depths ($F_{1,5}$ = 10.88, P <0.05; Table 2). The surface soils had 35% and 23% higher microbial biomass C than in the subsurface soil layers under the zero tilled and tilled treatments, respectively($F_{1,10}$ = 20.61, P <0.001). Microbial biomass nitrogen followed similar trends as that of microbial biomass C (Table 1; Tillage treatment: $F_{1,5}$ = 6.6, P <0.05; and Depth:$F_{1,10}$ = 13.29, P <0.05).

3.2.2 *Soil microbial functional diversity*

AWCD increased with incubation time, indicating the presence of active microbial flora in all treatments ($F_{4,119}$ = 433.18, P<0.001, Fig.2). Significantly higher AWCD values ($F_{1,23}$ = 29.03, P<0.05) were recorded for zero tilled soils compared to tilled soils. The surface layer had higher AWCD values in both treatments compared to the subsurface layer ($F_{1,23}$ = 27.47, P<0.05). PCA did not provide a clear separation of C substrate utilization betweeneither tillage treatments or soil depth.

3.2.3 *Soil enzymatic activities*

Zero tilled soils had 60% higher dehydrogenase activity thantilled soils when averaged across both surface and subsurface layers($F_{1,5}$ = 19.54, P<0.01) (Fig. 3a). The surface layer had greater dehydrogenase activity than the subsurface layer (Tillage treatment: $F_{1,10}$ = 148.08, P<0.001). Similarly, the activity of three extra cellular hydrolytic enzymes namelycellulase, xylanase and β-glucosidase washigher in zero tilled soilsthan tilled soil by 140, 38 and 28% respectively(Fig. 3b-d, $F_{1,5}$ = 21.98, P<0.01; $F_{1,5}$ = 8.34, P<0.05; $F_{1,5}$ = 14.28, P<0.05). The activities of these enzymes were greatest in surface soils (Depth: $F_{1,10}$ = 24.42, P<0.001; $F_{1,10}$ = 21.95, P<0.001; $F_{1,10}$ = 18.06, P<0.01 for cellulase, xylanase and β-glucosidase, respectively).

Of the two oxido-reductive enzymes studied, phenol oxidase activity was greater (26%) under zero tillage (Tillage treatment: $F_{1,5} = 31.49$, $P<0.01$) and activity was highest in the surface soil (Depth: $F_{1,10} = 30.27$, $P<0.001$). There was no significant effect of either tillage or depth on the peroxidase activity in soil.

To assess if the changes in enzyme activities were driven by either increased availability of carbon substrates or increased microbial biomass the impact of tillage and soil depth on soil enzymes were also calculated per gram of organic matter as well as the specific enzyme activity (per microbial biomass carbon in soil basis)..With regards to the specific enzyme activity, the tillage treatment did not significantly impact any of the enzymes we investigated (Supplementary Table 1). Enzyme activities expressed per gram of organic matter in the soilshowed very similar trends to those in Fig. 3, however, the tillage treatment was significant only for the cellulase ($F_{1,23} = 6.96$, $P< 0.05$) and dehydrogenase activity($F_{1,23} = 16.34$, $P< 0.01$).

3.3 Factors affecting carbon content in soil

The carbon content in soil was predicted by a multiple regression model ($F_{5,18} =32.9$, $P< 0.001$) including β-glucosidase (BG), dehydrogenase (DH), xylanase (X), soil moisture (M) and clay content in soil (Clay) which accounted for 90.1% of the variation. The optimal model for C is provided in the equation 4.

$$C\ (\%) = 0.981 - 0.00818BG + 0.1351DH + 0.3382X - 0.01462M + 0.01452Clay \quad (4)$$

In this model, the soil clay content (used as a descriptor of soil type) contributed to 19.1% of variation, estimated by dropping the parameter when fitted last from the model. The rest of the variation can be attributed to the soil enzymes and soil moisture availability (Figures 4a, 4b, 4c and 4d). Simple linear regression showed soil moisture on its own was not related to soil C

330 (P>0.05). The multiple regression analysis of greenhouse gases (GHGs) against different soil
331 enzymes and other properties showed no significant relationships.

332 **4. Discussion**

333 The higher soil C content found in zero tilled soils (9% over 7 years)in our study was
334 comparable to that shown previously (8% after 12 years; Ernst and Emmerling [33] and 16%
335 after 25 years;Plaza [34]. This enhanced C content in zero tilled soil has previously been
336 attributed to the retention of crop residues at surface and root biomass in the subsurface
337 layers[18, 35]and lower decomposition rates[36] which is supported by our CO_2 flux data,
338 which was lower under zero tillage.

339

340 The C protection in soil is also dependent on the form in which it is stored. In this study, zero
341 tilled soils contained a greater amount of aromatics and/or CH_2whichis a relatively recalcitrant
342 fraction of soil C [37]. Indeed, the absorbance bands which increased in zero tilled soils are
343 most likely the culmination of multiple substitution patterns around an aromatic ring
344 contributing to a single absorbance band(s), for example, mono- and meta-substituted rings
345 absorb in the region 720-680 cm^{-1}, thus would cumulatively reinforce the IR signal in this
346 region. If lignin is a major contributor to the recalcitrant fraction with a slow decomposition
347 rate, the absorption fingerprint of lignin at lower wavenumbers/longer wavelength (and other
348 related biopolymers) fits well with spectral data presented here [38, 39]. Accumulation of
349 aromatics under zero tillage may be due to the preservation of lignin during decomposition of
350 crop residues which are greater on zero tilled soils [40] or enhanced microbial stabilization of
351 organic materials [10].

352 The increased microbial biomass and activities (AWCD) observed in zero tilled soil may be
353 due to a more continuous supply of organic materials to soil microorganisms in the absence of

354 tillage [41]. Microbial intracellular and hydrolytic extra cellular enzymatic activities were also
355 higher in zero tilled soils, in parallel with previous findings [42, 43]while oxido-reductive
356 enzyme activities (phenoloxidase and peroxidase) were less strongly affected by zero tillage.
357 Acosta-Matinez et al. [18]attributed increased enzyme activities under non disturbed pasture
358 soil to either the presence of active microbial biomass, constituting intracellular enzymes
359 and/or to extracellular enzymes, which remained part of soil organic matter. Due to the lack of
360 disturbance in zero tilled soils, the biochemical environment is less oxidizing than in tilled soil
361 [43] which may result in a more stable pool of extracellular enzymes [44] explaining, at least
362 in part, the higher enzyme activities in zero tilled soils. Surface accumulation of crop residues,
363 and subsurface supply of organic materials through root biomass, could contribute to enhanced
364 enzyme activities in zero tilled soils. However, enzyme activities were enhanced in tilled soils
365 also when accounting for soil C content suggesting that enzyme activities in zero tilled soil
366 were stimulated by factors above and beyond total C availability. The enhanced enzyme
367 activities suggest microbial transformation of soil organic matter and plant residue is favored
368 in zero tillage systems.

369 Zero tillage reduced emission of CO_2, suggesting either that the activity of the microbial
370 community is reduced by zero tillage, through for example reduced porosity and lower
371 substrate availability, or that the microbial community is less stressed [45]and function more
372 effectively in zero tilled soils i.e. their respiration relative to their biomass is reduced. In our
373 study, zero tillage increased the soil C content, microbial biomass, soil enzyme activities and
374 decreased the metabolic respirational quotient of the microbial community. Furthermore, the
375 extracellular hydrolytic enzymes involved in C metabolism (cellulase, xylanase, β-glucosidase)
376 were all positively correlated with C content, as also observed by Katsalirou and co-authors or
377 cellulose and β-glucosidase[46]. As these enzymes act upon the polysaccharides in crop
378 residues and root biomass and convert them into soil humus and recalcitrant C in different soil

aggregates, this suggests the enhanced activity of these enzymes help sequester C in soil [19]. Together our data supports the notion that zero tillage can enhance soil C storage by reducing microbial CO_2 respirational losses, through reduced oxidative stress, and enhanced enzymatic transformation of organic material. We propose this mechanism together with the greater addition of crop residues associated with zero tillage are important drivers of the increased C storage under zero tillage in temperate regions[47].

Lignin and other complex organic compounds in plant residues are rate limiting in the later stages of litter decomposition and important for subsequent humification and sequestration of C in soil [48, 49]. Lignin degradation is brought about by oxidative enzymes such as phenol oxidase and peroxidase enzymes produced mainly by fungi. Increased activities of phenol oxidase and peroxidase in zero tilled soil are attributed to the absence of soil disturbance which allow fungal hyphae to make bridges between soil and crop residues [50]. The increased activities of phenol oxidase under zero tilled conditions in our study suggests zero tilled soils stimulated fungal activity which may aid C sequestration as fungal cell wall compounds such as chitin and melanin degrade slowly in soil[51].

In contrast to the increased enzyme activities, the biolog work did not suggest a shift in the functional diversity of the fast growing component of soil bacteria, which may indicatet hat the changes in enzyme activities reported here may be attributed to greater abundance of fungi in zero tilled soil. Reduced microbial functional diversity has previously been reported under tilled conditions in response to soil disturbance that adversely affects the soil organisms, e.g. tillage breaking up fungal hyphae[52]. Greater C sequestration in soil with higher clay content is most likely due to absorption of organic C to clay surfaces, entrapment of C in aggregates or encapsulation of organic C by clay particles [53]. Lower disturbance may also improve preservation of microbial products in stabilized micro and macro aggregates [53-55]. Indeed,

tillage mediated aggregate changes can lead to changes in carbon storage in soil, depending on soil texture[56].

Impacts by zero tillage on soil aggregation also appeared to influence CH_4 fluxes. Zero tillage has previously been found to increase CH_4 oxidation in intact soil cores with preserved soil structure as a methanotrophic community develops in undisturbed soil[3]. In contrast, the current study found greater CH_4 production in zero tilled soil from loose soil. This is most likely related to the type of aggregates created by zero tillage, as small aggregates tend to produce more CH_4[56]. Together, these findings suggest zero tillage may increase CH_4 production within aggregates but that the produced CH_4 is subsequently consumed by a more active methanotrophic community.

In conclusion, we found zero tillage strongly influenced the functioning of the microbial community as reflected by reduced respiration rates and greater enzyme activities. Furthermore, soil under zero tillage management accumulated greater amounts of total C and a greater proportion of aromatic C. Together, this shows that the functioning of the microbial community is highly responsive to zero tillage and that it may play an important role for the sequestration of C in temperate agricultural soils.

Acknowledgements

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Table 1
Site characteristics of the study sites

	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6
Location	Bourne 1	Bourne 2	Melton 1	Melton 2	Oakham-1	Oakham-2
Geographical coordinates	Lat. 52.4600° N Long. 0.2259° W		Lat. 52.7661°N Long. 0.8860° W		Lat. 52.6705° N Long. 0.7333° W	
Elevation (m)	28	58	54	43	75	94
Years in no-till management	7	7	7	7	7	7
Cropping activity in tilled site	Wheat	Wheat	Wheat	Wheat/Peas	Wheat	Wheat
Cropping in no-tilled site	Wheat	Wheat	Wheat	Wheat/Oil Seed Rape	Wheat	Wheat
Soil texture	Clay	Clay	Clay	Silty clay	Silt loam	Silty clay loam
World reference base classification [57]	Luvic Gleysol		Eutric Vertic Stagnosol		Calcaric Leptosol	

Table 2

Total C, total N, microbial biomass C (MBC), microbial biomass N (MBN) at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils*.

Tillage	Depth (cm)	Total C (%)	Total N (%)	MBC (mg kg^{-1} soil)	MBN (mg kg^{-1} soil)
Zero tilled	0-10	1.53±0.14	0.301±0.04	650±104	110.4±20
	10-20	1.32±0.14	0.202±0.02	425±69	66.4±15
Tilled	0-10	1.41±0.16	0.175±0.02	425±66	61.9±11
	10-20	1.18±0.10	0.149±0.02	328±67	46.3±11

*Mean±Standard Error (n=6)

Table 3

F statistic from analysis of variance (ANOVA) for the absorbance at different wave numbers from the FTIR spectra.

Wave number (cm^{-1})	Tillage	Depth	Tillage × depth	Functional group
2925	1.99 ns	1.29 ns	0.09 ns	CHn, Aliphatics
2850	0.13 ns	1.93 ns	0.07 ns	CHn, Aliphatics
1801	0.0 ns	0.49 ns	0.30 ns	C-O, C=O or N
1799	0.0 ns	0.5 ns	0.27 ns	C-O, C=O or N
831	5.13 ns	0.55 ns	0.15 ns	CH_2, Aromatic
829	5.16 ns	0.52 ns	0.25 ns	CH_2, Aromatic
827	5.17 ns	0.51 ns	0.34 ns	CH_2, Aromatic
825	5.32 ns	0.50 ns	0.48 ns	CH_2, Aromatic
823	5.55 ns	0.48 ns	0.62 ns	CH_2, Aromatic
821	5.85 ns	0.50 ns	0.76 ns	CH_2, Aromatic
819	6.1 ns	0.58 ns	1.02 ns	CH_2, Aromatic
761	2.06 ns	0.55 ns	2.58 ns	Aromatics
759	2.01 ns	0.66 ns	2.70 ns	Aromatics
711	10.11*	10.19**	0.69 ns	Aromatics
709	8.23*	9.06*	0.75 ns	Aromatics
671	0.45 ns	0.76 ns	0.93 ns	Aromatics
669	0.40 ns	1.1 ns	0.78 ns	Aromatics
665	0.88 ns	1.09 ns	0.09 ns	Aromatics
651	0.51 ns	3.57 ns	1.73 ns	Aromatics
649	0.36 ns	3.75 ns	2.07 ns	Aromatics

NS: non-significant.
*** $p<0.001$.
** $p<0.01$.
* $p<0.05$.

Table 4

CO_2 flux, CH_4 flux and N_2O flux at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils.Mean±Standard Error is shown (n=6).

Tillage	Depth (cm)	CO_2-C flux	CH_4-C flux	N_2O-N flux	qCO_2
		µg m^{-2} g^{-1} h^{-1}	ng m^{-2} g^{-1} h^{-1}	ng m^{-2} g^{-1} h^{-1}	µg CO_2-C per microbial biomass carbon in mg g^{-1} soil per hour
Zero tilled	0-10	3.78±0.67	1.098±0.23	1.03±0.64	5.94±0.47
	10-20	2.98±0.43	0.593±0.16	0.8±0.22	7.46±0.94
Tilled	0-10	6.29±1.01	0.388±0.34	0.71±0.26	16.97±3.84
	10-20	5.17±1.23	0.021±0.24	0.46±0.20	17.15±3.75

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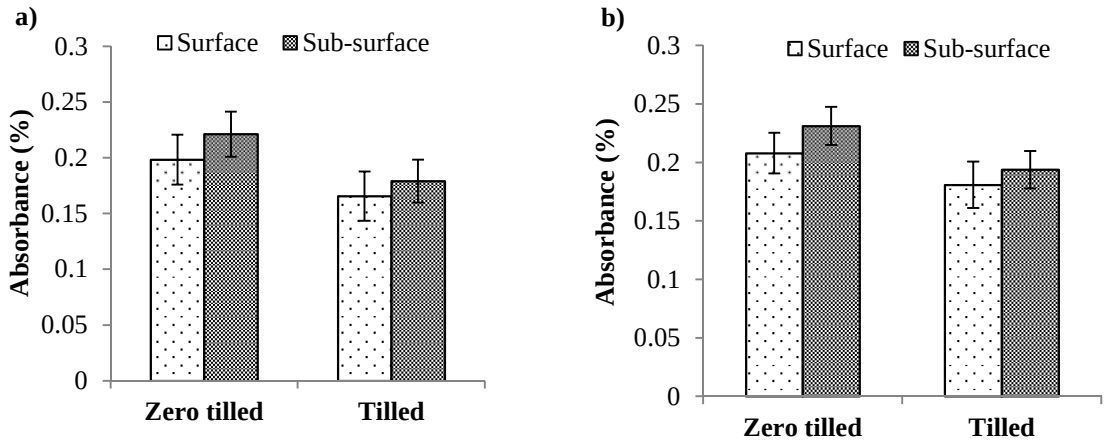

Fig. 1. Absorbance values at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils at wave nmbers (a) 711, (b) 709.

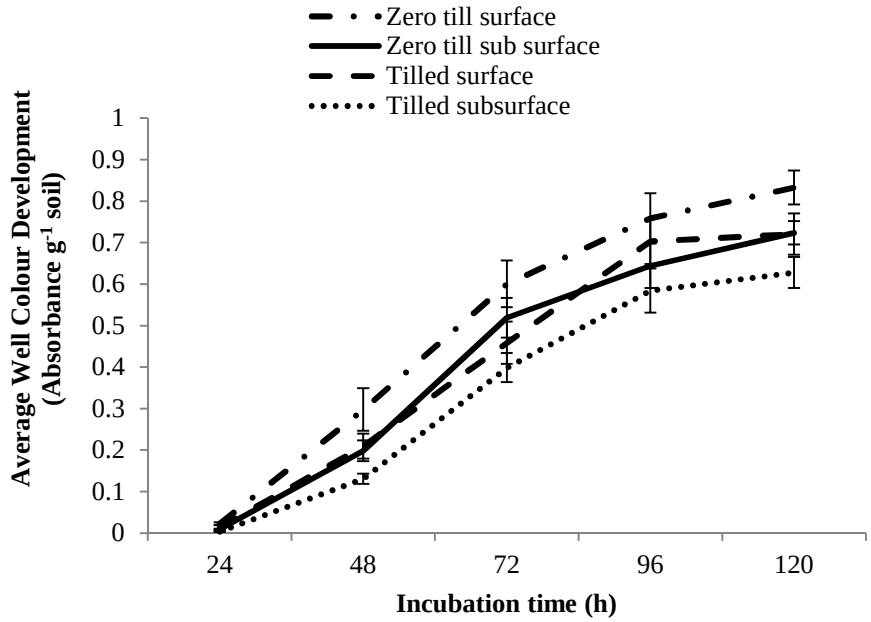

Fig. 2. Average Well Colour Development (AWCD) obtained by Biolog ecoplates. Error bars indicate standard error of means (n=6).

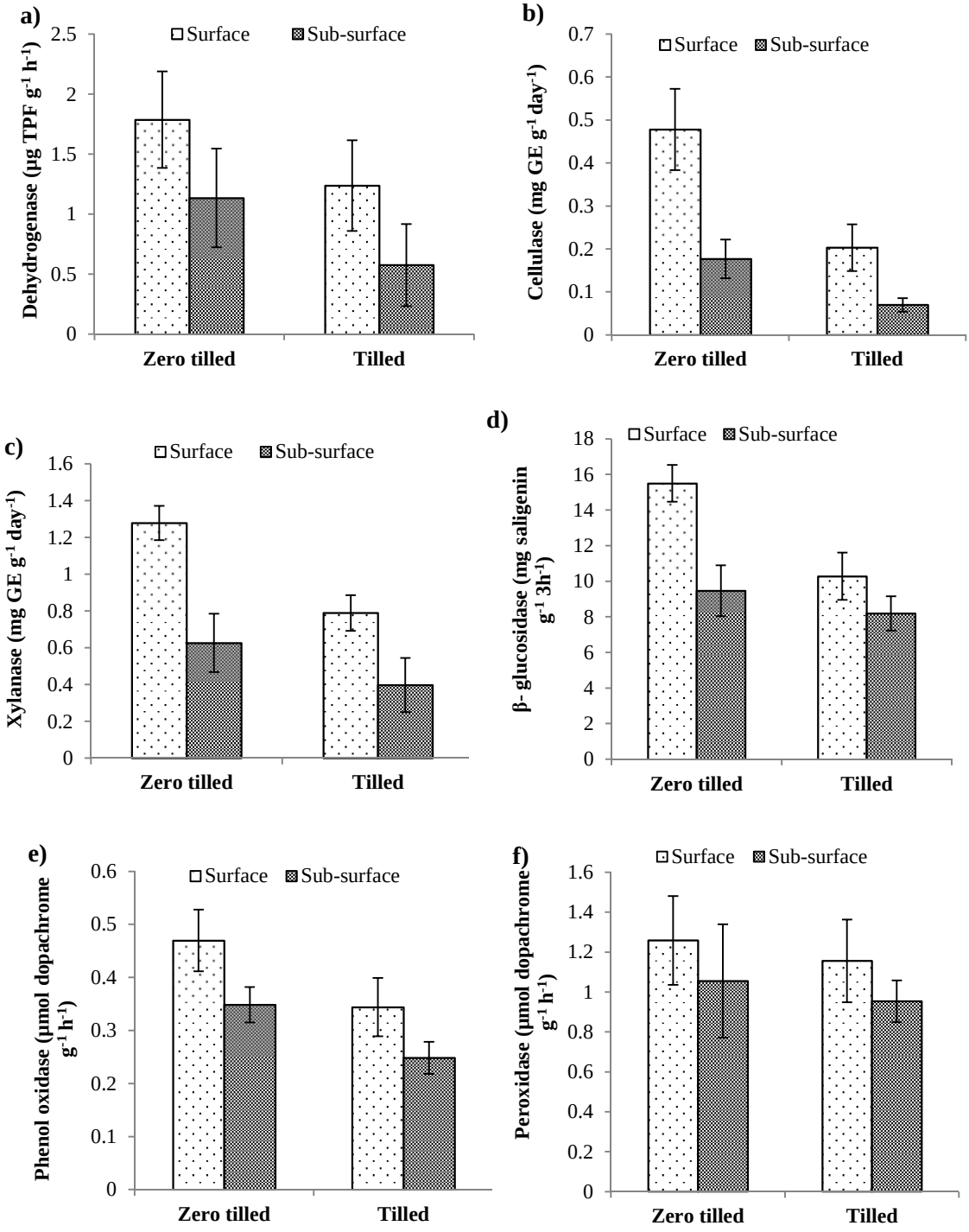

Fig. 3. Soil enzymes at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils; (a) dehydrogenase, (b) cellulase, (c) xylanase, (d) β-glucosidase, (e) phenol oxidase and (f) peroxidase.

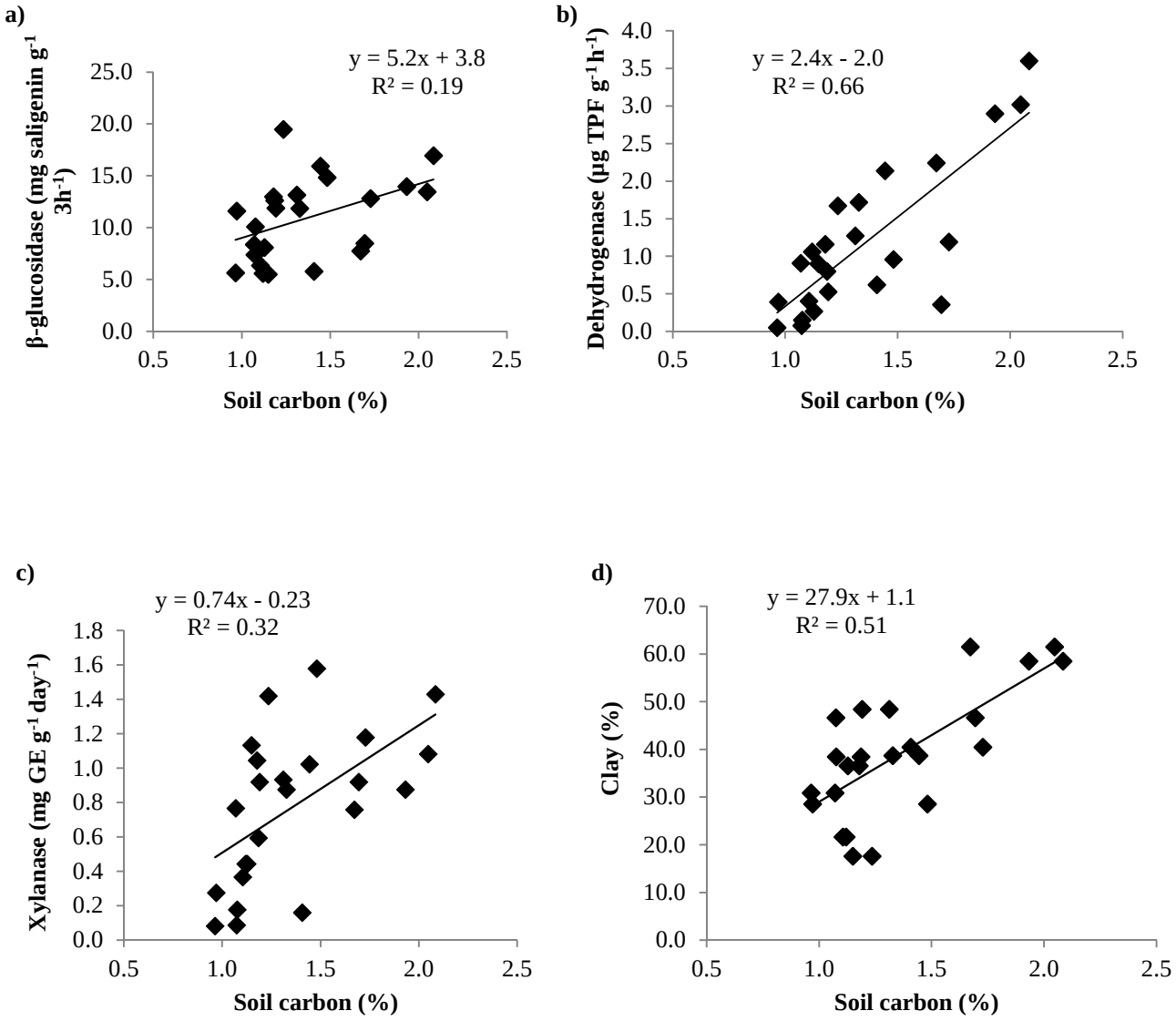

Fig. 4. Illustration of relationships between soil biophysical properties and soil C (a) β-glucosidase and soil C content; $F_{1,22}$=5.26, P<0.05 (b) dehydrogenase and soil C; $F_{1,22}$=41.91, P<0.001 (c) xylanase and soil C; $F_{1,22}$=10.27, P<0.01 (d) soil clay content and soil C;$F_{1,22}$=22.89, P<0.001.

Supplementary table 1

Soil enzymes at surface (0-10 cm) and subsurface (10-20 cm) layers under zero tilled and tilled soils on per microbial biomass carbon basis(Mean±Standard Error is shown).

Tillage	Depth (cm)	Dehydrogenase (µg TPF mg^{-1} microbial carbon g^{-1} soil h^{-1})	Cellulase (mg GE mg^{-1} microbial carbon g^{-1} soil day^{-1})	Xylanase (mg GE mg^{-1} microbial carbon g^{-1} soil day^{-1})	β-glucosidase (mg saligenin mg^{-1} microbial carbon g^{-1} soil $3h^{-1}$)	Phenol oxidase (µmol dopachrome mg^{-1} microbial carbon g^{-1} soil h^{-1})	Peroxidase (µmol dopachrome mg^{-1} microbial carbon g^{-1} soil h^{-1})
Zero tilled	0-10	4.47±2.09	0.90±0.28	2.37±0.52	27.44±4.89	0.85±0.17	2.27±0.46
	10-20	2.98±1.65	0.43±0.09	1.45±0.34	24.15±4.24	0.97±0.17	2.84±0.79
Tilled	0-10	2.68±0.86	0.53±0.15	2.20±0.62	26.85±4.97	0.93±0.20	3.18±0.76
	10-20	1.85±1.10	0.25±0.06	1.11±0.32	28.91±5.70	0.92±0.18	3.67±0.89
Tillage		2.25 ^{ns}	4.62 ^{ns}	1.58 ^{ns}	0.42^{ns}	0.02 ^{ns}	1.1 ^{ns}
Depth		16.34**	6.96*	3.63 ^{ns}	0.06 ^{ns}	0.90 ^{ns}	2.92 ^{ns}
Tillage x depth		1.34 ^{ns}	0.46 ^{ns}	0.07 ^{ns}	1.18 ^{ns}	1.49 ^{ns}	0.02 ^{ns}

F statistic from ANOVA is given.

Ns- non significant, *** $p<0.001$, ** $p<0.01$, * $p<0.05$.