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Summary

Models of landscape carbon sequestration focus primarily on changes in land use and give little indication of modifications to carbon flux due to differing land management practice within the same category. In the agricultural category, for example, reseeding and ploughing practices vary. Ploughing modifies flux rates in agricultural grasslands by disruption of soil and removal of photosynthetic material. There is limited literature on the effects of differing practices. We present data of flux rates based on deep and minimum tillage overseeding and controls. Using a randomised block design we measured flux from 20 plots using a Perspex chamber and portable infrared gas analyser (IRGA). Our data suggests that loss of photosynthetic material from destruction of the grass sward temporarily changes grasslands from sinks to sources. For example flux from deep ploughed treatments reduced from mean of 0.82 ±0.11 to -1.85±0.2 g CO_2 m^{-2} hr^{-1} while control plots ranged from -1.88±0.11 to -2.41±0.2 g CO_2 m^{-2} hr^{-1}.

Key words: CO_2 flux, carbon, sink, source

Introduction

Models of land-based carbon sequestration account for land use type, i.e. differences between land use categories, but exclude variation of management practices within each category. For example, grasslands in temperate regions require periodic tillage and reseeding in order to maintain a healthy sward (Drewer *et al.*, 2017), but there are a paucity of data on the effects of tillage and reseeding, and their frequency, on local carbon fluxes (Willems *et al.*, 2011). Failure to account for potentially large variations in local carbon fluxes is likely to result in biased and inaccurate estimates of CO_2 emissions at the national level. For example, in the United Kingdom (UK), land-based carbon sequestration estimates are produced using 'Land Use, Land Use Change and Forestry' (LULUCF). Assessments which are modelled based on Intergovernmental Panel on Climate Change (IPCC), Tier 1 approaches at regional scales

(Moxley *et al.*, 2014; Thomson, 2017) but excluding variation within regions due to differing management practices.

Ploughing results in major disruptions to soil, leading to CO_2 loss (Palm *et al.*, 2014; Soane *et al.*, 2012; Powlson *et al.*, 2011; Lal, 2004). Ploughing aerates soil and allows the incorporation of added nutrients to maintain high harvest yields (Drewer *et al.*, 2017). In non-ploughed systems, soil structure is denser, soils have a higher moisture content (Sheehy *et al.*, 2013), and carbon is protected from decomposition due to physical protection within soil aggregates (Ogle *et al.*, 2012). Ploughing also results in a loss of photosynthetic material for a time, affecting Gross Primary Production (GPP). Re-establishment of a grass sward, after approx. 100 days, can return flux levels to pre-ploughed levels (Hadden & Grelle, 2017). There are varying management practices for grassland reseeding including: conventional ploughing, where soil is inverted from a depth of 20–30 cm, minimum tillage, which disrupts only topsoil (~5 cm depth) and overseeding, where new seed is sown into an existing sward (Schulz *et al.*, 2014).

We performed a field experiment to compare post-ploughing Net Ecosystem Exchange (NEE) and Ecosystem Respiration (R_e) time-series, measured using a portable infra-red gas analyser, between various management practices. We predicted that differing ploughing treatment blocks would exhibit significant differences in their carbon flux resulting from structural changes to soil; which, at cumulative large-scales, are likely to impact the accuracy of national carbon emission estimates.

Materials and Methods

Study site

The experiment was conducted at the Agri-Food and Biosciences Institute (AFBI), Hillsborough research farm in County Down, Northern Ireland (latitude, longitude: 54^o 27'N, -6^o 04'W). The site was in intensive grassland production using perennial Italian rye-grass (*Lolium perenne*) and had been used for sheep production in the previous year. The soil was a clay loam / silty clay loam formed over a Triassic red sandstone mixed till or SWG1 (Higgins *et al.*, 2012). (UK soil inventory). The site had not been ploughed for approximately 15 years prior to the experiment.

Experimental design

A randomised block design was developed where each of four experimental treatments were replicated five times (20 plots in total); each plot was 2 m × 1 m. Treatments consisted of T1: Conventional ploughing (spraying off, ploughing to a depth of 20 cm), power harrowing, rotavated, sowing seed, and rolling), T2: Minimum tillage (spraying off, rotavated to a depth of 5 cm), power harrowing, sowing seed, and rolling), T3: overseeding (drilling-seed), and T4: Controls (unmanaged plots of existing sward). T1 and T2 sprayed with a glyphosphate-based herbicide (160 mL of Roundup^{TM} in 10 L water) three weeks before treatment application. All plots (except the controls) had applications of 29.62 g plot^{-1} of Nitro chalk (27% N), 6.92 g plot^{-1} of grass seed and 40 g plot^{-1} of agricultural lime after initial treatment application.

Measuring carbon flux

Flux measurements were quantified using a large, 50 L, chamber made from transparent perplex. Sampled air from inside the chamber headspace passed through an attached infra-red gas analyser (EGM 4, PP-systems Hitchin UK) and flux determined as the difference between in and out flow concentrations (Welles *et al.*, 2001). Internal air was mixed with a small fan to ensure equal distribution of CO_2 (Pumpanen *et al.*, 2004). Net Ecosystem Exchange (*NEE*) was measured with a transparent chamber allowing photosynthesis to occur in the chamber headspace. Ecosystem Respiration (R_e) was measured using the same chamber with a dark cover blocking light suppressing drawdown by photosynthesis. R_e measurements were made immediately after *NEE* measurements; each measurement period was 120s. Flux readings were randomised between plots and taken between 10:00 and 14:00 on each measurement day, to minimize diurnal effects on flux in accordance with IPCC good practice guidelines. Measurements commenced on 15/09/2015 taken three times a week until 29/10/2015 after which frequency was weekly until 22/12/2015 when measurements ceased. Eight infrequent measurements were taken from 10/02/1016–22/04/2016 to investigate flux over a longer time frame.

Environmental variation

Time-series of air temperature and soil temperature (°C) at 10 cm and 30 cm depths and soil moisture (%) were obtained from an adjacent weather station, approx. 10 m from the study site, connected to the environmental change network (see http://www.ecn.ac.uk/ for details). Continuous variables were recorded hourly and daily averages obtained. Data was then compared based on days of flux measurement.

Soil core samples were collected using a hydraulic soil core to a depth of 10 cm. For each sample, carbon (C) and nitrogen (N) (%) was measured by dry combustion chamber (Leo Trumac, Stockport, UK). Phosphorus (P) using an automated continuous flow wet chemistry analyser (Skalar San plus, Holland), and potassium (K) content using Flame Photometer, (Sherwood Scientific model 410). Soil bulk density measurements were also taken for all plots but after flux measurements had been completed. A surface sample of undisturbed soil was taken to a depth of 5 cm. The sample was weighed, dried until constant mass and re-weighed to determine percentage (%) moisture content. The dry weight was then divided by the volume of the sample container to obtain a measure of the bulk density.

Grass canopy height was measured using a sward measure. Five random points within each plot were selected and height measured when a transparent platform made first contact with any grass structure. Measurements were averaged for each plot. Grass height was recorded on three occasions (26/05/2016, 22/07/2016 and 17/08/2016) to investigate any treatment effects.

Statistical analysis

Flux measurements of *NEE* and R_e were analysed as the dependent variables by treatment using restricted (residual or reduced) maximum likelihood (REML) using a power model due to the time-dependent nature and the non-regularity of the data. Variation in weather conditions were analysed using Principal Components Analysis (PCA) reducing air temperature, soil temperate (at both 10 cm and 30 cm) and soil moisture content to two axes. Block*Plot was fitted as a Random Factor, to the REML model, PC1, PC2 and Time as covariates. To test effects from loss of photosynthetic material the factor ‘Photosynthesis’ was created that split the dataset into two parts: 1) post-treatment up to 100 days and 2) after 100 days. Representing pre and post sward re-establishment. The interaction of this factor and treatment was also fitted as Photosynthesis*Treatment. Residual plots demonstrated the data to be normally distributed (Gaussian). Fisher’s Least Square Difference (LSD) was used to identify post-hoc differences between treatment means. Grass and soil metrics were compared between treatments using a one-way ANOVA. All statistics were performed using Genstat 16th Edition (VSN International Ltd.).

Results

REML analysis showed a significant difference in treatment and across time for NEE but not for R_e (Table 1). Treatments T1 and T2 (were previous sward was destroyed) became net sources of CO_2 (mean flux T1 0.82±0.11 g CO_2 m^{-2} hr^{-1}, T2 0.88±0.11 g CO_2 m^{-2} hr^{-1}) for approx. 100 days post ploughing after which time both returned to being net sinks (mean flux T1 -1.85±0.2 g CO_2 m^{-2} hr^{-1}, T2 -1.84±0.2 g CO_2 m^{-2} hr^{-1}) (Fig.1). T3 and T4 were net CO_2 sinks for the duration of the study period (mean flux first 100 days T3 -1.61±0.2 g CO_2 m^{-2} hr^{-1}, T4 -1.88±0.2 g CO_2 m^{-2} hr^{-1}; mean flux after 100 days T3 -2.18±0.2 g CO_2 m^{-2} hr^{-1}, T4 -2.41±0.2 g CO_2 m^{-2} hr^{-1}). Treatments T1 and T2 did not differ from one another (Fisher's LSD *post-hoc* P>0.05) and T3 and T4 did not differ from one another (P>0.05) but T1 and T2 did differ from both T3 and T4 (P<0.05) (Fig. 2). The differences observed were driven by post-treatment effects in the first 100 days with little difference observed throughout the rest of the study period. Environmental components (See supplementary material) were significantly influential for both NEE and R_e (Table 1). PCA suggested temperature (Air, and Soil, 30 cm and 10 cm) and soil moisture accounted for much variation (50%), with rainfall and solar radiation secondary components (26%). The loss of photosynthesis was not directly significant, however when crossed with treatment it was for NEE, this was not the case for R_e (Table 1).

Experimental treatments did not influence soil carbon ($F_{df=3}$=0.64, P=0.598), nitrogen ($F_{df=3}$=0.32, P=0.812), phosphorus ($F_{df=3}$=0.75, P=0.538) or potassium ($F_{df=3}$=0.39, P=0.761) though variance was typically greater for most soil nutrients within ploughed treatments T1 and T2 than unploughed treatments T3 and T4 (Fig. 3a–d). Soil bulk density did vary significantly between treatments (F_{df3}=5.50, P=0.013) being significantly evaluated in the ploughed treatment T1 (Fisher's LSD *post-hoc* P<0.05) whilst the unploughed T4 controls had the lowest values (Fig. 3e).

Mean grass canopy height did not differ between treatments ($F_{df=3}$=0.38, P=0.767) nor did grass dry matter ($F_{df=3}$=0.78, P=0.77). The longest grass lengths were recorded in the unploughed T4 control plots (Fig. 3f).

Discussion

Net Ecosystem Exchange (*NEE*) was strongly affected by tillage with ploughed treatments being a CO_2 source during the first 100 days post-ploughing (which occurred during autumn in the current study) before returning to being a net CO_2 sink similar to our unploughed and control treatments. These results appear to be driven by the loss of photosynthetic material (i.e. the sudden destruction of the standing grass crop) rather than differences in flux rates. CO_2 release has been observed after soil disruption at shallow (5 cm) and deep (20 cm) soil horizons at similar situations in Ireland and Scotland, with fluxes returning to pre-ploughed levels approximately 2 months (*ca*. 60 days) after ploughing (Willems *et al.*, 2011; Helfter, 2015) . A fourfold increase in CO_2 release has previously been observed due to post-ploughing (Merbold *et al.*, 2014). Re-establishment of vegetative grass cover due to reseeding subsequently converts ploughed plots to CO_2 sinks several months after ploughing.

Ecosystem respiration (R_e) was unaffected by ploughing. Fig. 1b suggests there is a decline in flux rates before rising again. This coincided with the winter period thus, R_e decreases during winter before increasing the following spring (Hadden & Grelle, 2017; Flanagan & Johnson, 2005). When variation due to time was taken into consideration no significant difference was observed. Ploughing is recommended when environmental conditions are sub-optimal for soil microbial activity to reduce respiration and thus CO_2 release (Rutledge *et al.*, 2014). Hadden & Grelle (2017) observed higher respiration rates in set-aside land when compared to cultivated land during spring with differences ascribed to higher vegetation biomass adding organic matter to the soil increasing decomposition by microbial activity. Our results suggest, that in the short-term where ploughing occurs in autumn, subsequent low winter temperatures (and thus lower root and microbial activity) reduce CO_2 release which resulted in no differences in *Re* between ploughed and unploughed treatments.

Failure to account for management practices i.e. the type of tillage and the frequency of its repeated use (for example, regular reseeding) on carbon fluxes within temperate grassland production systems may result in biased and inaccurate estimates of carbon sequestration during National-level inventories. Merbold *et al.* (2014) report that a 10-year rotation for grass was insufficient to restore half of the CO_2 lost due to the just one ploughing event in Switzerland. Meta-analyses suggest carbon stocks accumulate in the upper layers of non-ploughed (no till) systems and whilst ploughing impacts upper layers it has little impact at lower depths (Powlson *et al.*, 2011; Angers & Eriksen-Hamel, 2008) . Below the ploughed layer, carbon is more recalcitrant (Rasse *et al.*, 2006). Thus, the treatments applied in the current study are unlikely to have affected carbon storage deeper than the depth of the plough. It may be difficult to detect the impact of short-term perturbations on soil carbon pools if large background deposits of carbon are present; with impacts taking up to 10 years to detect depending on the magnitude of the perturbation (Smith, 2004).

Many studies have reported a reduction in soil bulk density with ploughing (Osunbitan *et al.*, 2005), however, we report the opposite effect with ploughed treatment T1 Deep ploughing (spraying off, ploughing to a depth of 20 cm), power harrowing, rotavation and sowing seed) significantly increasing soil bulk density. T1 was the only treatment to affect soil below 5 cm (the measurement level of bulk density). Ploughed treatments were rolled, adding weight and compacting soil, after establishment, possibly accounting for the change in bulk density. Mahmoudi *et al.* (2015) observed an increase in bulk density with increased depth of tillage. The soils in the current study had a high clay component, which can show a higher bulk density after ploughing than non-ploughed soils or soils with a low or no clay component (Laine *et al.*, 2017; Necpálová *et al.*, 2014; Ji, 2013).

Soil nutrient content and subsequent grass canopy height was unaffected by tillage during the current experiment perhaps due to the application of agricultural lime and fertiliser during reseeding which may have masked any loss of nutrients. Ploughing soils allows for greater root penetration by reducing resistance (Mahmoudi *et al.*, 2015). The small sampling time may also play a role. Grasslands are reseeded only rarely, approx. once every 10 years (Merbold *et al.*, 2014) it is conceivable that the grass in treatment 4 would degrade over time showing less growth potential than the reseeded treatments. This hypothesis would need investigated further.

The duration of the experiment was short (<1 year) with data collection during autumn, winter and spring coinciding with low biological activity with few *NEE* and R_e measurements taken during winter. As flux rates, particularly for NEE, were correlated with environmental variables it would be necessary to construct a longer time series (1 year) with measurements of soil temperature and moisture in plot as opposed to in an adjacent plot as here.

Nevertheless, we provide clear evidence supporting the hypothesised changes in flux post-ploughing of temperate grasslands where the system moved to being a carbon source for approximately 100 days before returning to being a carbon sink. These changes are likely attributed to the loss of photosynthetic material rather than effects of ploughing *per se*.

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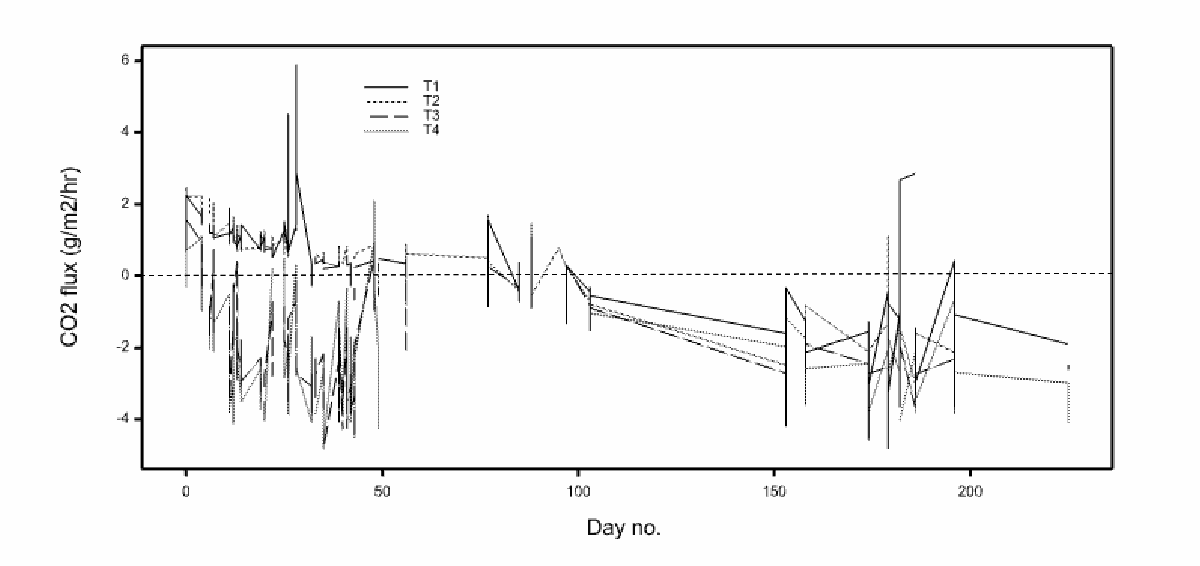
Figures

a)



b)

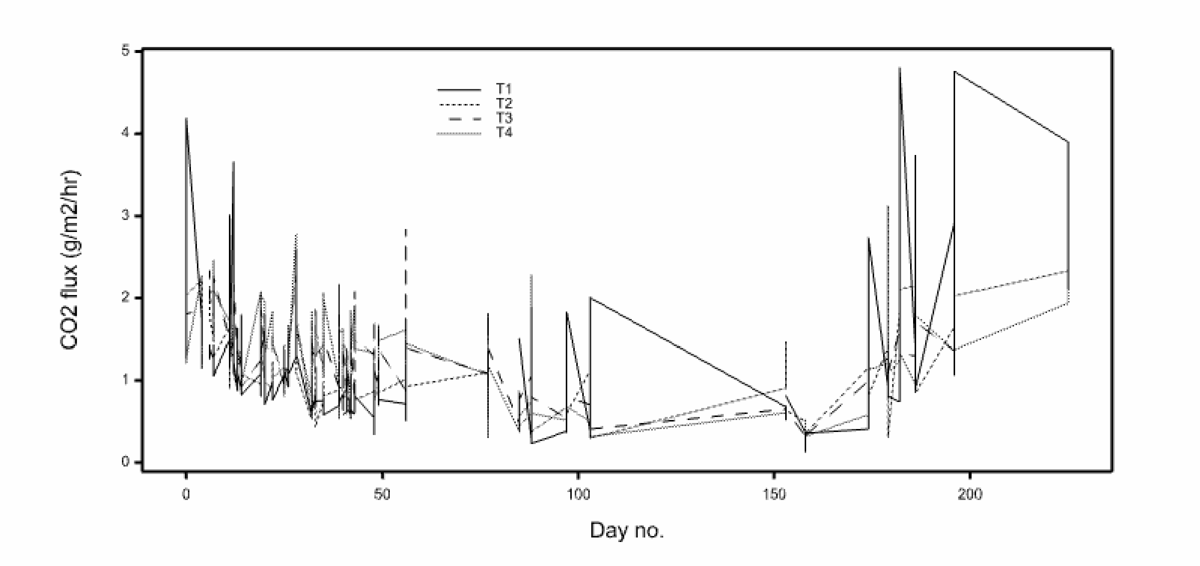


Fig. 1 Time-series throughout the experiment for fluxes in **a)** Net ecosystem exchange (NEE) and **b)** ecosystem respiration (R_e) across treatment blocks. Straight dashed line represents 0 flux. The convention is positive figures represent a loss of CO_2 from the ecosystem and negative figures represent a sequestration of CO_2.

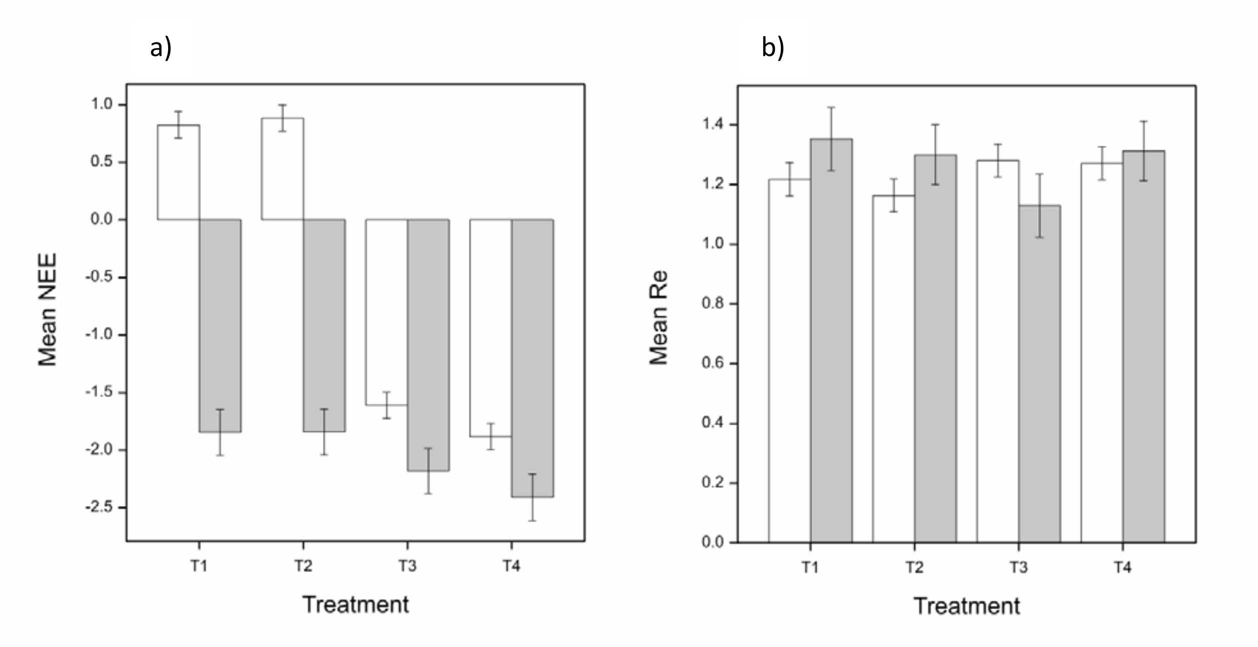


Fig. 2 Bar charts showing a) mean NEE pre and post canopy establishment and B) Mean R_e pre and post conopy establishment. Error bars are standard error. White bars represtnt pre-100 days and grey bars post 100 days.

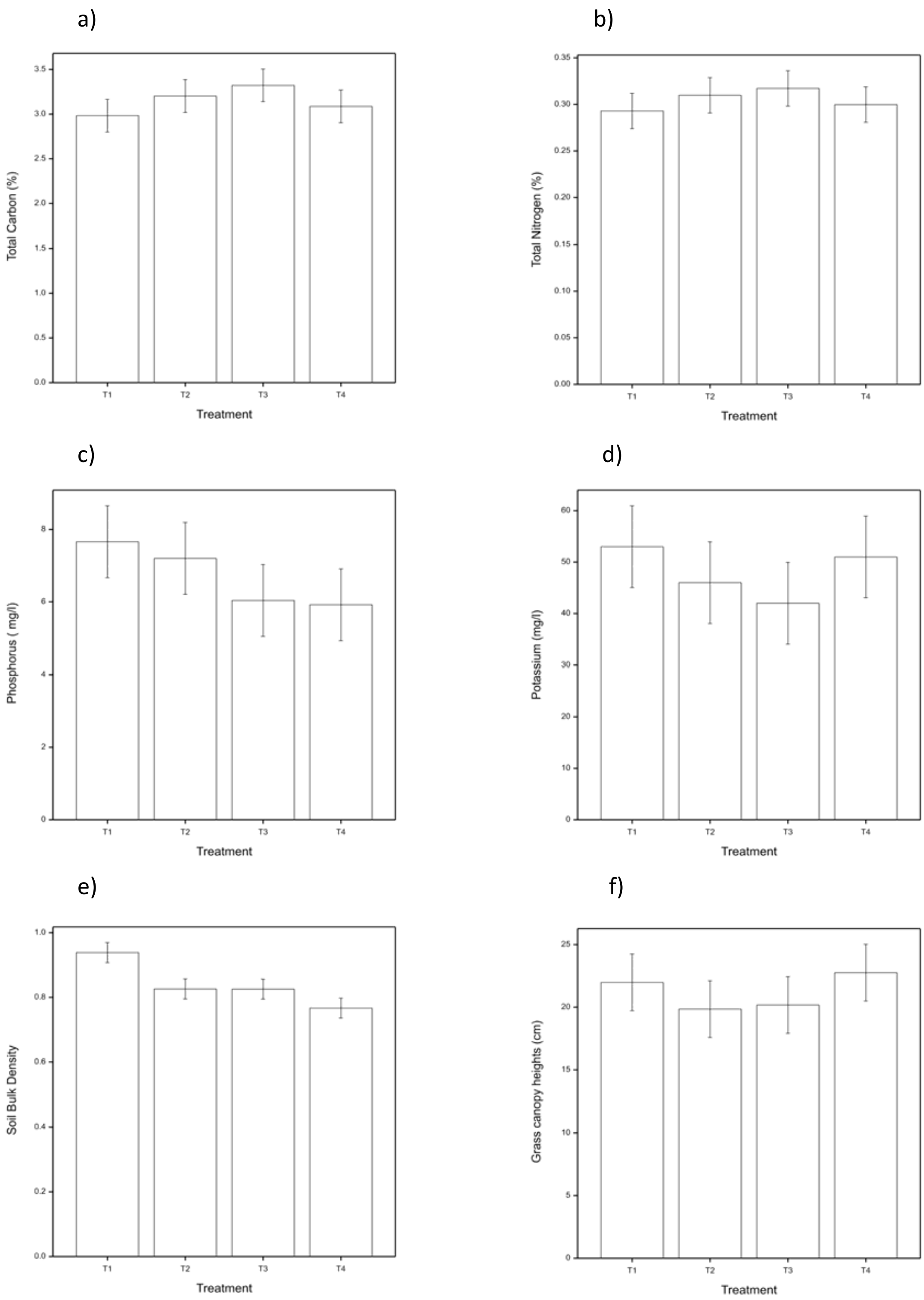


Fig.3 Barcharts showing the mean for **a)** soil carbon (%), **b)** nitrogen (%), **c)** phosphorus (mg/l), **d)** potassium (mg/l) content, **e)** bulk density (with LSD scores) and **f)** grass canopy height (cm) after re-establishment of sward across four treatment blocks: T1: Deep ploughing (spraying off, ploughing to a depth of 20cm), power harrowing, rotavation, sowing seed, and rolling), T2: Minimum ploughing (spraying off, subsoiling to a depth of 5cm), power harrowing, sowing seed, and rolling), T3: overseeding (drilling-seed), and T4: Controls (unmanaged plots of existing sward). Error bars are Standard error.

Table 1 Restricted (or residual, or reduced) maximum likelihood (REML) model outputs for a) NEE and b) R_e. Block*Plot was fitted as a Random Factor.

Model / Variable	**F**	**β ± s.e.**	**n.df**	**d.df**	**p**
a) *NEE*					
Treatment	152.85	Factorial	3	628.5	<0.001
Time	224.80	-0.354 ± 0.149	1	628.5	<0.001
PC1	5.15	0.213 ± 0.095	1	628.7	0.024
PC2	5.48	0.101 ± 0.053	1	628.9	0.020
Photosynthesis	2.45	Factorial	1	628.6	0.118
Treatment*Photosynthesis	30.47	Factorial	3	628.7	<0.001
b) R_e					
Treatment	0.73	Factorial	3	703.1	0.532
Time	0.21	0.458 ± 0.064	1	703.5	0.649
PC1	157.01	0.536 ± 0.042	1	703.4	<0.001
PC2	118.38	-0.229 ± 0.022	1	703.2	<0.001
Photosynthesis	0.28	Factorial	1	703.1	0.600
Treatment*Photosynthesis	1.46	Factorial	3	703.1	0.223

Online Supplementary Materials

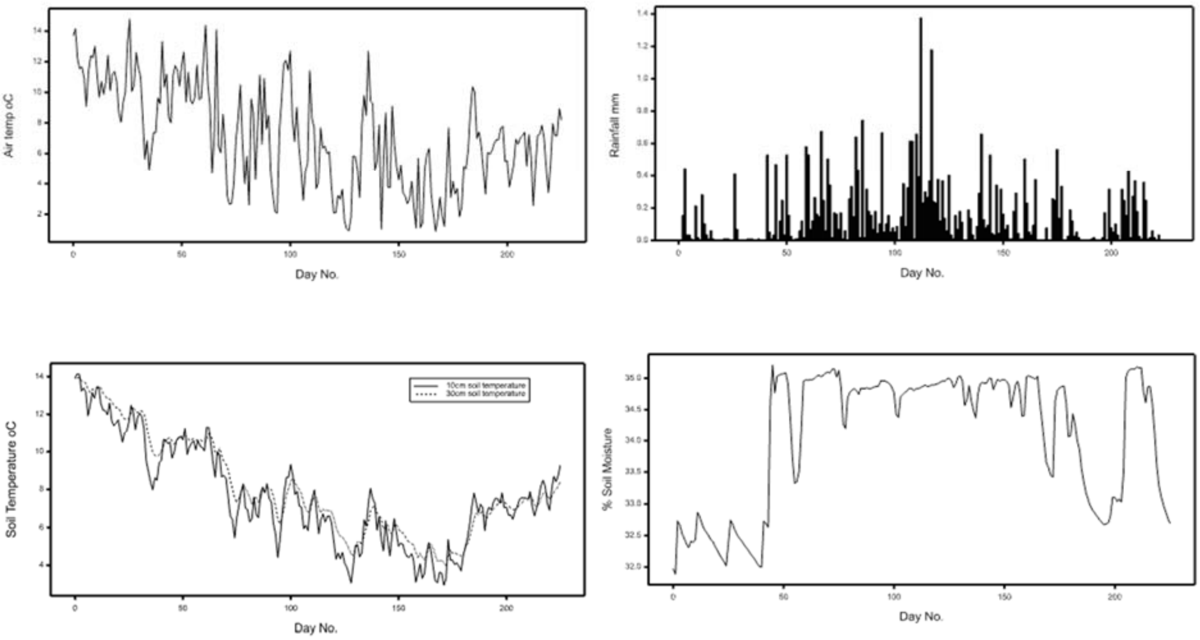


Fig. S1 Times-series showing environmental for **a)** air temperature, **b)** rainfall, c) soil temperature at 10 and 30cm depths and **d)** soil moisture content. Atmospheric variables were measured at adjacent weather station.

Table S2. Summary statistics for soil analysis

Element	Treatment	Mean	S.D.	S.E.
C	T1	2.28	0.33	0.15
	T2	3.20	0.65	0.29
	T3	3.32	0.12	0.05
	T4	3.09	0.35	0.16
N	T1	0.29	0.04	0.02
	T2	0.31	0.07	0.03
	T3	0.32	0.02	0.01
	T4	0.30	0.04	0.02
P	T1	7.66	2.59	1.16
	T2	7.20	3.20	1.43
	T3	6.04	0.96	0.43
	T4	5.92	1.36	0.61
K	T1	53.00	23.61	10.56
	T2	46.00	8.22	3.67
	T3	42.00	5.70	2.55
	T4	51.00	24.60	11.00