SOW14-GPLER-SP23-PFR-MB: Accelerated soil C sequestration through targeted use of full inversion tillage when renewing permanent pastures and grasslands

Lysimeter experiment to quantify impact of FIT on Irish soils

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Abstract

Deep ploughing, or full inversion tillage offers a strategy to both protect soil organic carbon stocks that have built up over previous decades, whilst providing the capacity for a 'new C sink' to be established. However, key questions remain as to how this renovation may impact on yield and also key components of the ecosystem C cycle. This study utilised a C tracer approach in order to attempt to characterise key leverage points by which deep ploughing could enhance the C cycle. Gross primary productivity (GPP0 was observed to be lowest in the non-renovated pastures. Soil respiratory losses were found to be impacted by tillage intensity, with the ratio of GPP:TER and auto- to heterotrophic respiration also influenced, possible due to the fact that microbial activity and biomass is constrained under deep-ploughing soil conditions. In conclusion, deep ploughing may positively impact net CO_2 sink activity by both increasing GPP, but more especially reduce TER and particularly heterotrophic respiration.

1.Introduction

Globally, soils constitute the largest terrestrial carbon (C) sink. Around 1500 Pg C is with the top 3 m estimated to contain 3,200 Pg C as soil organic carbon (SOC). Soil in the 1 m (1472 Pg C) and 2 m (2400 Pg C) depths contain at least three times the amount of CO_2 in the atmosphere (~830 Pg C) (Batjes, 2014). Grassland soils contain 303 Pg C to a depth of 1 m which is around 20% of the world's total soil carbon stock (Stockmann et al., 2015). Soil C sequestration is currently targeted as a strategy to off-set increases in atmospheric CO_2 emissions. For example, the '4 per mille' initiative aims to increase soil C concentration in agricultural soils by 0.4% yr^{-1}, producing a net C sink of 1.2 Pg C yr^{-1}. Smith et al. (2007) estimate a maximum potential of 1.6 Pg C yr^{-1} for enhanced agricultural management to mitigate CO2 emissions to the atmosphere. Increases or decreases in SOC could, therefore, have large impacts on atmospheric carbon dioxide concentrations (CO_2).

The C sequestration potential of a soil is dependent on its ability and capacity to stabilize SOC. Its potential is estimated as the difference between existing SOC and the theoretical SOC maximum amount of carbon that can be stabilized under optimal conditions, irrespective of management, climate and carbon inputs (Stewart et al., 2008; Castellano et al., 2015). Soils that are C saturated have little capacity to increase SOC, whereas under-saturated or C deficient soils have greater potential for

accruing SOC. The SOC can become stabilized via physical, chemical or biochemical mechanisms, the formation of organo-mineral complexes (chemical) in the fine (silt and clay) fraction is considered the most important mechanism (Baldock & Skjemstad, 2000; Beare et al., 2014; Hassink et al., 1997; McNally et al., 2017).

Grasslands occupy 67.1% of the total land area in Ireland (ca. 3 Mha) and sequester significant amounts of C into plant biomass and soils. Previous studies have reported that Irish grassland mineral soils are under saturated, with SOC concentrations ranging from 3.2-6.3% in the 0-50 cm depth (Kiely et al., 2010; Xu et al., 2011). However as permanent pastures in Ireland are rarely renovated, with less than 4% reseeded annually the capacity to increase C inputs and soil C storage may limited under current management strategies. While non-disturbance has led to a large build of recalcitrant carbon, many grasslands may have reached SOC equilibrium (dependent on soil, climate and wider management) and have retarded levels of productivity (Torres-Sallan et al. 2017). In a recent study, Simo et al. (2019) found that up to 40 t ha^{-1} of stable SOC is contained below the 30 cm depth. Torres-Sallan et al. (2017) found that 42% of the stable SOC in subsoil (below 30 cm) is located in microaggregates plus silt and clay compared to 16% in the topsoil (0-30 cm), in soils subject to clay illuviation. Their results indicate that the stability of the SOC stocks may vary significantly between soil types, and subsequently, how management will influence long-term SOC storage.

Historically the use of minimum tillage, conservation or non-inversion tillage has been utilised in order to minimise soil disturbance, prevent aggregate disruption and thus preserve soil organic carbon (SOC) stocks (Six et al. 1999; Abdalla et al. 2013). However, some studies have observed that minimum tillage merely concentrates SOC accumulation in the top 10-15 cm and that when SOC levels were integrated over the full plough depth, no difference in total OC was observed compared to inversion tillage (Angers et al. 2008). Alternately, minimum tillage may enhance SOC preservation in hotter, more arid regions by enhance water holding capacity and preventing erosion (Jacobs et al. 2010).

An alternative strategy to minimum tillage is to inversion plough the carbon-rich topsoil deep enough so as to protect it from aerobic decomposition. This will lift subsoil to the surface, essentially providing new C sink capacity, with new carbon inputs gradually increasing the amount of SOC within the new soil profile (Beare et al, 2020). Inversion tillage (FIT) (≥30 cm depth) followed by reseeding is a common technique for sward renewal in Ireland. However, deep inversion past 30 cm in uncommon. In this scenario, a short-term C loss is conceded to maintain the production potential of the pasture. Once off or infrequent deep ploughing can increase SOC stocks via two processes: (i) bury carbon-rich topsoil and possibly slow the rate of decomposition, and (ii) transfer carbon-deficient soil to the surface to increase C input by plants. FIT has been previously shown to increase C stocks by 3-25% after 50 years (cropland) (Alcantra et al., 2016) and ~18% (0-30 cm) after four years (pasture) (Calvelo-Pereira

et al., 2018). In the latter study, the authors found that the accumulation of new C from reseeding (ΔC +13.9 Mg ha^{-1}), rather than the conservation of buried C (ΔC -1.3 Mg ha-1), was the principal process influencing SOC stocks following FIT.

Consequently, few researchers have analysed the impact of grass species following FIT. Multi-species swards often contain deep rooting, as well as higher proportions of nitrogen-fixing clovers, which may cause an increase or decline in soil C storage. More diverse pastures can have greater root biomass with increased C input to soils compared to ryegrass-clover pastures (McNally et al., 2015). Calvelo-Pereira et al. (2016) detected no net loss of C at depth two-years after soil inversion in lysimeter treatments sown with cocksfoot (*Dactylus glomerata* L.), compared to a ryegrass treatment. Rutledge et al. (2017) reported potential C losses following pasture renewal in ryegrass and mixed species swards, due to increased respiration from decomposing tissues in the old sward. However, these losses were compensated by increased GPP. The effects of deep rooting species on SOC are, for now, inconclusive (Whitehead et al., 2018).

In order to assess the proportion of CO_2 emitted from the inverted vegetation layer as well as quantifying the input of this buried C into soil aggregates, an isotope tracing experiment was established. This study aimed to assess whether topsoil OC is stabilised under varying tillage intensities and plant species mixtures via ^{13}C tracer experiment utilizing lysimeters under optimal environmental conditions (Balesdent et al. 1988, Keuskamp et al. 2013). This method depends on the plants that providing a source of carbon with an isotopic composition ($\delta^{13}C$) that is different to the soil. A simple isotopic mixing model can then be used to calculate the percentage of new C entering the soil from the labelled plant material. It is also possible to derive additional kinetic information that describes the rate of C accumulation, turnover of recently added C and pools of C within the total C stock.

Ecosystem CO_2 balance or Net Ecosystem Productivity (NEP) is a measure of the balance between CO_2 uptake by photosynthesis and release by respiration. Gross primary productivity (GPP) is defined as the total amount of CO_2 fixed per unit area over a specific time period. Conversely total ecosystem respiration (TER) is the sum total of CO_2 emitted due to auto- and hetero-tropic respiratory processes per unit area. CO_2 flux measurements in conjunction with the isotope tracing were measured using chamber measurements in order to quantify the impact of deep inversion tillage on Net Ecosystem Productivity (NEP) as well as gross component fluxes GPP and TER. The major concern of this technique is that burying nutrient rich topsoil may adversely impact GPP and hence yields, although recent work has shown that deep inversion ploughing could positively impact grass and crop yields (Calvelo-Pereira et al. 2020; Beare et al., 2020).

The objective of this study was to assess the impact of full inversion tillage by a) quantifying SOC conservation at depth with a combined ^{13}C labelling and C_4 decomposition approach and b) assessing the impact of deep inversion tillage on ecosystem CO_2 fluxes.

2. Materials and methods

2.1. Experimental site and lysimeter setup

Intact vegetation-soil lysimeters were extracted from the Cricket field site at Johnstown Castle Research Centre (52.296, -6.507681) on the 18th February 2019. The soil is classified as a loam (45% sand, 35% silt and 20% clay) with a sandy loam soil texture in the upper 0-15 cm and gleyic features in the subsoil horizons (78+ cm). Soil characteristics are detailed in Table 1. Each lysimeter (25 cm diameter, 60 cm length) was placed into a cast iron shoe with a lid and hammered into the soil with a backhoe loader. When retrieved from the soil, the exposed ends of the lysimeters were covered with aluminium trays and sealed with duct tape. The lysimeters were then stored at a growth room facility under ambient environmental conditions.

Table 1: Soil physical and chemical properties associated with lysimeters

Soil Property	Value
GPS coordinates	54°45'127N, 6°04'5785W
Drainage[a]	Imperfectly
Soil pH	5.6
Soil texture[b]	Clay Loam
Sand (%)	45.1
Silt (%)	34.6
Clay (%)	20.3
Bulk density[c]	0.79
Soil Total carbon (%)	5.16
Total nitrogen (%)	0.45
Soil Loss on ignition (%)	12.5
Cation exchange capacity (cmol(+) kg^{-1})	25.4

[a]Drainage Classification was based on the soil associations from the Soil map of Ireland (Gardiner and Radford, 1980)
[b]Soil texture classification determined using LandIS portal © Cranfield University, UK.
[c] Stone-free Bulk density sampled to 10cm

Fig. 1 The lysimeter extraction process before (left) and after (right). Lysimeters were housed in the cast iron shoe and drilled into the soil, before being retrieved with the JCB digger (see description above).

At least eight lysimeters were maintained in pristine condition to act as permanent pasture (PP) controls. For those remaining, twenty-four lysimeters were sprayed with Glyphosate (0.6 l ha^{-1}) on the 28th February. The lysimeters were back-filled with petrolatum during vegetation senescence. The petrolatum was heated on a camping stove until molten. A 50 ml plastic syringe was used to inject petrolatum into any gaps present between the soil and the lysimeter. The petrolatum was allowed to settle and harden before the lysimeters were placed upright once more. Back-filling was performed to prevent preferential flow of water along the sides of the column walls.

After senescence, minimum tillage (MT), conventional tillage (CT) and deep tillage (DT) was simulated on selected lysimeters. For the MT treatment, the upper 2 cm of soil was removed, sieved to 10 mm and repacked into the soil column. For CT, soil was removed in 5 cm increments to a depth of 25 cm (0-5, 2-10, 10-15, 15-20 and 20-25 cm). For DT, soil was removed in 5 cm increments to a depth of 40 cm (0-5, 2-10, 10-15, 15-20, 20-25, 25-30, 30-35 and 35-40 cm). Full soil inversion was simulated whereby, for example, the top 0-5 cm replaced the 35-40 cm layer in the DT treatments, and so on, until each soil layer was repacked. The two lower depths of CT (15-20, 20-25 cm) and DT (30-35, 35-40 cm) were sieved to 10 mm to simulate the break-up of large aggregates after ploughing, typically done by one/two passes with a roller.

Before the soil was repacked, end-caps were attached to the base of each lysimeter which were subsequently housed in a custom-built timber frames (Fig. 2). First, the end caps and hydrodare pipe were threaded with braided fire rope. A glue gun was used to seal the fire-rope to the inner-surface of the end cap (Fig. 2). The function of the fire rope was to act as a wick for permeating water by mimicking soil tension under field conditions. Installing hydrodare pipes onto the end-caps will also allow dissolved organic carbon/nitrogen to be sampled from leached water.

Next, the lysimeters were inverted whilst maintaining the soil in place. Soil sieved to 4 mm was placed above the inverted lower soil depth (Fig. 2) before the end-cap was fitted around the lysimeter. Thirty-two lysimeters were then housed in two 4x4 timber frames (16 per grid). The experimental design was randomised with 2 replicate tilled treatments (MT, CT, DT) and four replicate PP treatments per timber frame. After setup, all lysimeters were watered to field capacity.

The lysimeters were reseeded on the 27th August 2019 as a (i) monoculture and (ii) a mixed-species sward. The grasses used were: (i) perennial ryegrass (*Lolium perenne* cv. AberChoice and cv. AberGain) and (ii) perennial ryegrass (*L. perenne* cv. AberChoice and cv. AberGain), white clover (*Trifolium repens* cv. AberHerald (small) and cv. AberAce (medium), red clover (*Trifolium pratense* cv. AberChanti), timothy (*Phleum pratense*), plantain (*Plantago major* cv. Tonic) and chicory (*Cichorium intybus* cv. Puna II) (Table 2). Each lysimeter was reseeded at double the standard seeding rate (105 kg ha^{-1}). Fertiliser (Calcium Ammonium Nitrate) was applied on 23 September 2019 at a rate of 50kg N ha^{-1}. The PP plots were assumed to contain a species proportion of ≥90% perennial ryegrass with ≤10% white clover. Plants were harvested on the 30th October 2019.

Fig. 2 Various stages of the lysimeter setup: gasket with neoprene lining (top left), inverted lysimeter with 4 mm sieved soil (top right), end-cap + hydrodare pipe with lysimeter prior to inversion (lower left) and the braided fire rope fanned out within the end cap (lower right).

Table 1. Species composition for the monoculture and mixed-species sward treatments

	Monoculture	Mixed-species
Lolium perenne	100%[a]	40%[a]
Trifolium repens		20%[b]
Trifolium pratense		7%
Phleum pratense		15%
Plantago major		8%
Cichorium intybus		10%

[a] 60% AberChoice: 40% AberGain

[b] 50% AberHerald: 50% AberAce

A canopy chamber was constructed using acrylic materials (Fig. 4). Firstly, holes of 26 cm diameter were engraved into a 10 mm opaque acrylic sheet. These horizontal sheets were fitted around the lysimeters and rested on plastic gaskets encircling the lysimeters. Several 60mm x 120mm' timber lengths were drilled to the corners and centre of the main timber frame to support this horizontally laid

sheet. Transparent 8 mm acrylic sheets were cut to size and used as the side panels for the chamber enclosure (Fig. 4). The ends were sealed together with a transparent polyolefin primer (Tec7) and when set, slid onto the opaque acrylic base sheet. One transparent 8 mm acrylic sheet lined with 7 mm neoprene was mounted onto the side panels as the 'roof' on the enclosure. The canopy chambers will be used to create an airtight headspace volume for each pulse labelling event.

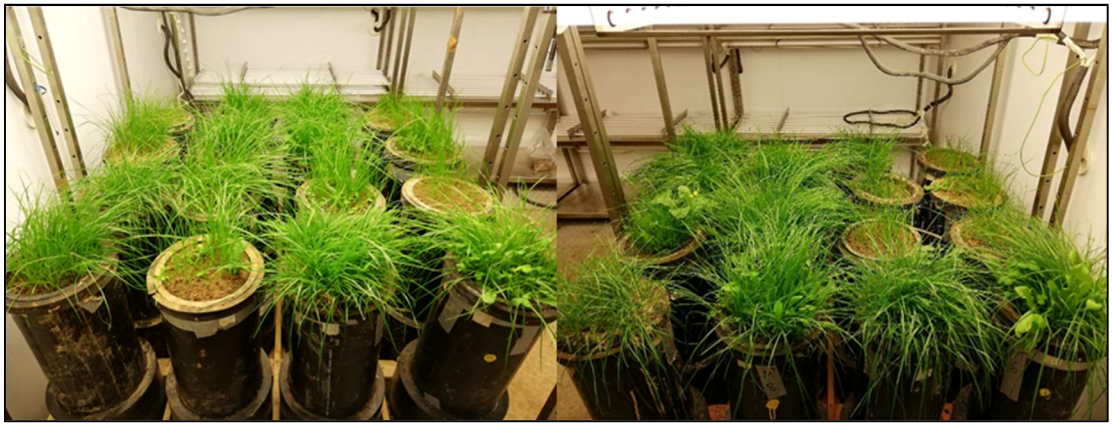

Fig. 3 Plant regrowth in the lysimeters 36 days post-reseeding.

Fig. 4 Completed chamber construction with the engraved 10 mm opaque acrylic laid horizontally over the lysimeters with the 8 mm acrylic side panels and roof.

2.2. Quantifying SOC conservation

Fresh maize (*Zea mays* L.) leaves were collected, dried at 60 ºC, ground and sieved to 0.5mm. Approximately 2.5 g of tissue was placed into empty non-biodegradable nylon bags (Lipton green tea EAN: 87 22700 05552 5) and sealed along the seam with a fabric glue gun. During the soil repacking stage, maize litter bags were buried at 5 cm (MT, CT and DT), 25 cm (CT and DT) and 40 cm (DT) depths from the top of the lysimeter in the tilled treatments. As maize uses the C_4 photosynthetic pathway, it has a distinct isotopic signature ($\delta^{13}C$ = -12‰) relative to plant roots and bulk soil ($\delta^{13}C$ =

-26‰). This technique was used in order to assess the incorporation of litter inputs into soil organic carbon. A two-pool mixing model based on a mass balance approach will be applied to elucidate if the change in $\delta^{13}C$ of SOC fraction is due to increased C input by plant roots or decomposing vegetation.

$^{13}CO_2$ labelling

The plants were grown at 20 ºC under full light (circa 500 µmol m^{-2} s^{-1} of photosynthetically active radiation) with a photoperiod of 20 h light and 4 h darkness. Plants were watered daily to field capacity. Relative humidity will be maintained at 70% with vapour pressure deficits ranging from 1.75 to 2.0 kPa. All plots received NPK and lime additions under standard management practice for pasture. A biomass harvest was taken five weeks prior to labelling. Harvested biomass was not significantly different between different tillage treatments or between Lolium and multispecies swards (745 ± 44 g m^{-2} and 709 ± 65 g m^{-2} for Lolium and multi-species respectively).

To generate $^{13}CO_2$ labelled air, sodium carbonate (99% atom $Na_2{}^{13}CO_3$, Merck) was chemically converted to $^{13}CO_2$ by acidification with citric acid ($C_6H_8O_7$) producing CO_2 and H_2O as the end-products. Using a syringe, citric acid solution was injected into a Duran bottle containing 0.5 g sodium carbonate. The CO_2 liberated from the reaction was collected in a tedlar bag and subsequently injected into the acrylic chamber at pulse labelling events. Headspace CO_2 was sampled using a gas tight syringe inserted into a septum incorporated into the side of the chamber following introduction of the label. Labelling has been performed every 48 hours for a 8 week period. Data is shown for the first labelling event. Headspace $\delta^{13}C$ was +51.4 ‰ ± 2.5‰. Carbon dioxide concentration within the enclosure was monitored using a multi-gas photoacoustic infra-red gas analyser (INNOVA 1412, LumaSense, DK). When CO_2 concentrations fell below 350 ppm, enriched sodium carbonate was be added to increase the CO_2 once more.

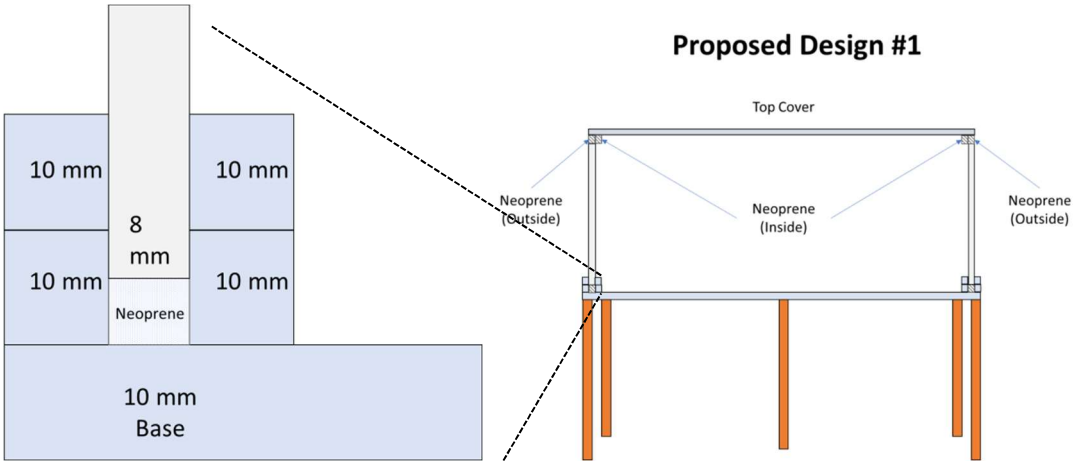

Fig. 5 Design to ensure airtight enclosure along the edges of the acrylic base (left) and the enclosure design (**137cm x 137cm x 40 cm**). Holes of 27 cm diameter will be cut into the acrylic base to fit over

the lysimeters. This base will be resting on neoprene attached to a plastic gasket sealed around the circumference of each lysimeter. The structure was supported by vertical timber frames (right).

Flux measurements

Flux measurements were performed prior to and following each labelling events. Data is shown for the first labelling event. Net ecosystem productivity (NEP) was measured by placing a clear polycarbonate chamber over each lysimeter and sealed with neoprene gaskets. This chamber was coupled to a photoacoustic (INNOVA 1412, LumaSense, USA) in a closed, dynamic configuration. Chambers were then covered with opaque material and ecosystem respiration (TER) was measured. Gross primary production was calculated as

$$GPP = NEP - TER \quad (1)$$

Gas samples were removed periodically during these measurements for isotope analysis and placed in exetainers with the CO_2 concentration of each sample recorded (Labco, High Wycombe, UK). Smaller chambers were placed on areas of bare soil in the lysimeters and connected to an infra-red gas analyser (Li-6400, Licor, Lincoln, Nebraska) in order to measure belowground or soil respiration. Post flux measurement, gas samples were removed over time and stored in exetainers for isotope analysis. All CO_2 fluxes were calculated from the change in CO_2 concentration over time.

Isotope measurements

Immediately after CO_2 trapping, shoot, root and soil samples were taken, dried at 60^{o}C for 48 hours, ground in a ball mill and analyzed for C% and N using a Carlo Elba 1108 elemental analyzer connected to a Thermo Scientific Delta Plus XP continuous flow isotope ratio mass spectrometer at the Godwin Laboratory, University of Cambridge. The carbon isotope ratio of gas samples were analysed using a Thermo Scientific 253 Plus in dual inlet mode in order to gain improved precision.

The isotopic signature of 'ecosystem and soil respiration' ($\delta^{13}C_{TER}$ and $\delta^{13}C_{SR}$) was calculated using the Keeling Plot approach, a two-member mixing model (air from the atmosphere and air from the ecosystem, Keeling 1958). Using the regression of measured $\delta^{13}C$ versus the inverse of the CO_2 mixing ratio, the isotopic signature of both ecosystem and soil respiration can be calculated as the y-intercept of the regression.

Ecosystem respiration was subsequently partitioned between above- and below-ground respiration using two methods. Firstly, partitioning was performed by subtracting soil respiration from ecosystem respiration. Secondly, using an isotope partitioning approach whereby the stable isotope signal of leaves and soil respiration can be used, with the end-member being total ecosystem respiration. The two source mixing model was applied as per Phillips and Gregg (2001). The carbon isotope ratio of soil respiration

could also be used to partition this net flux between auto- and hetero-trophic components in a similar fashion by exploiting the difference in isotope ratio between roots and soil.

Geometric mean regressions (GM model II) were used and uncertainties were reported as standard deviation (Webb et al. 1981). Outliers were excluded with an iterative residual analysis (samples with a residual of larger than three times the SD were removed).

3. Results

3.1. *Plant biomass*

Following reseeding, biomass accumulation was initially higher for the MT plots and decreased with tillage intensity in the CP and DP plots ($p<0.01$). Plant biomass in the MT ryegrass (6.40 ± 1.0 t DM ha^{-1}) and mixed species (5.96 ± 1.0 t DM ha^{-1}) plots were similar to the PP plots (7.05 ± 0.67 t DM ha^{-1}). Biomass yields in the ryegrass reseeded MT plots were at least 1.95 to 2.48 times greater than both grass mixtures of the CP and DP plots (Fig. 6, $p<0.05$). Grass species mixture had no effect on biomass accumulation within each tilled plot. However, one year after renovation, there were no significant differences between tillage treatments or between sward types, although the total biomass was lowest for the non-renovated pasture. In addition, measured rates of photosynthesis were lowest for the non-renovated treatment.

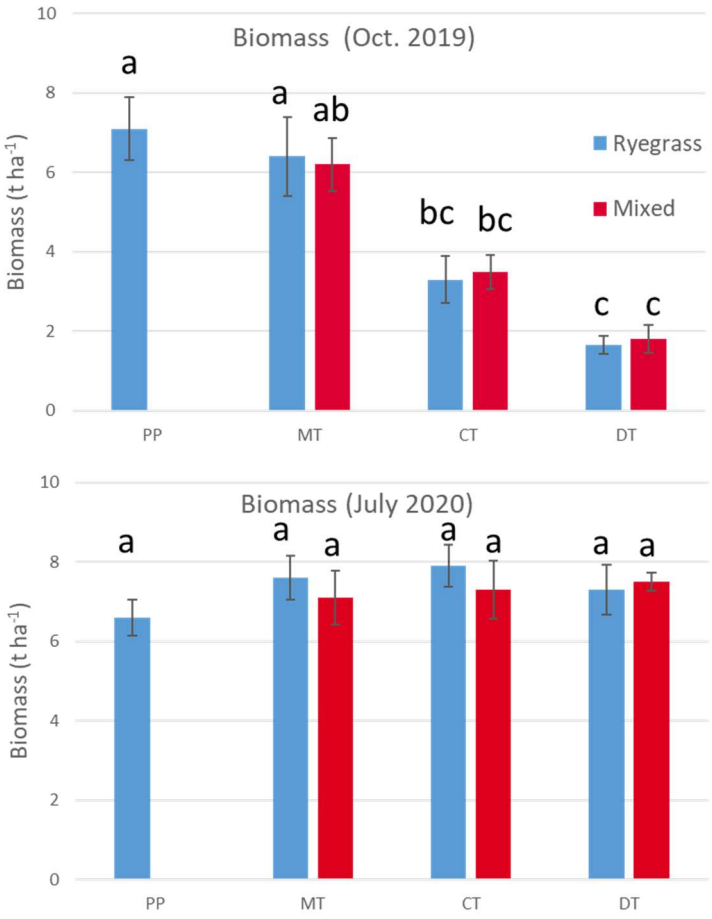

Fig. 6 Plant biomass in October 2019 (64 days post reseeding) and July (1 year after reseeding) in the minimum tillage (MT), conventional tillage (CP), deep tillage (DT) for ryegrass and mixed species, plus the permanent pasture ryegrass control (PP). Different letters above the vertical error bars (standard error of the mean) indicate significant differences between treatments ($p<0.05$).

Darkened chambers were used to estimate TER for all tillage types and sward types. Furthermore, the isotope ratio of both ecosystem respiration and soil respiration could be used to assess whether any of the C4 maize material was either a) being respired or b) had been incorporated into SOC. Keeling plots were generated from the relationship between $\delta^{13}C_{TER}$ and isotope concentration (Fig. 7). There were no significant differences between treatments for either parameter, although there was a trend whereby $\delta^{13}C$ in both ecosystem and soil respiration in the conventional-ploughed lysimeters was between 1.2‰ and 2.4‰ higher than other cultivation treatments (-24.9‰ for CT compared to, on average -26.8‰ for other treatments). This may be due to the fact that the shallower ploughing could result in higher microbial activity due to higher soil aeration than in minimum tillage treatments and also greater substrate availability as SOC levels were higher in the upper layers compared to the deep ploughed treatments (see field report). In addition, microbial biomass in upper soil layers following deep ploughing have been shown to be significantly lower (circa 80% lower) compared to the control, which would also retard decomposition (Alcantara et al. 2016).

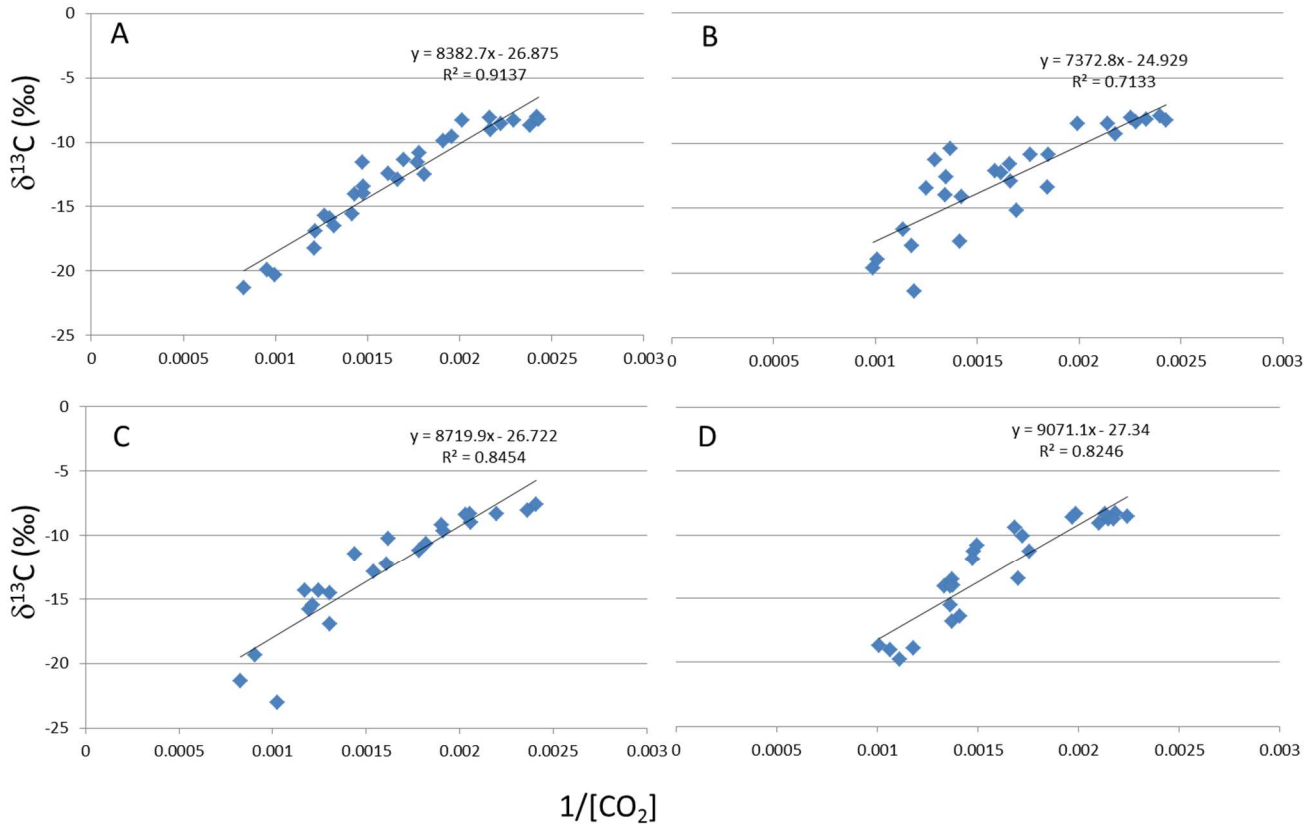

Fig. 7: Regressions between $\delta^{13}C$ and the inverse of CO_2 concentration for A) Deep ploughed, B) Conventional ploughed, C) Minimum tilled and D) non-renovated lysimeters

Significant differences in NEP values were observed between both tillage treatment and sward type, with values ranging between 268 mg C m^{-2} hr^{-1} for deep-ploughed *Lolium perenne* to 137 mg C m^{-2} hr^{-1} for unrenovated *L. perenne* swards ($p < 0.05$; Figure 8). NEP was significantly lower in the conventional ploughed treatments compared to the other cultivations, particularly the deep ploughed treatment (Figure 8A). This lower NEP in conventional-ploughed species mixtures was attributed to lower GPP, with values ranging from 302 – 344 mg C m^{-2} hr^{-1} (Figure 8B). However, for Lolium treatments, reduced NEP was attributed to higher ecosystem respiration in the conventional ploughed lysimeters, where values ranged from 219 to 240 mg C m^{-2} hr^{-1}, compared to a mean value of 198 mg C m^{-2} hr^{-1} and 202 mg C m^{-2} hr^{-1} for the deep till and minimum tillage treatments respectively (Figure 8C). Extremes in both GPP and TER/soil respiration were observed in the deep ploughed treatments. The high NEP was observed for DP *Lolium* was driven by high GPP (Figure 8B), whilst the relatively high NEP for multispecies mixtures under DP was driven by very low TER (mean value = 153 ± 35mg C m^{-2} hr^{-1}). Lower TER values following deep ploughing is consistent with previous findings, where decomposition and microbial biomass were reduced (Alcantara et al. 2016). *Lolium* performed better in the lysimeters due to better establishment, particularly after first harvest, when light levels were increased (Figure 6).

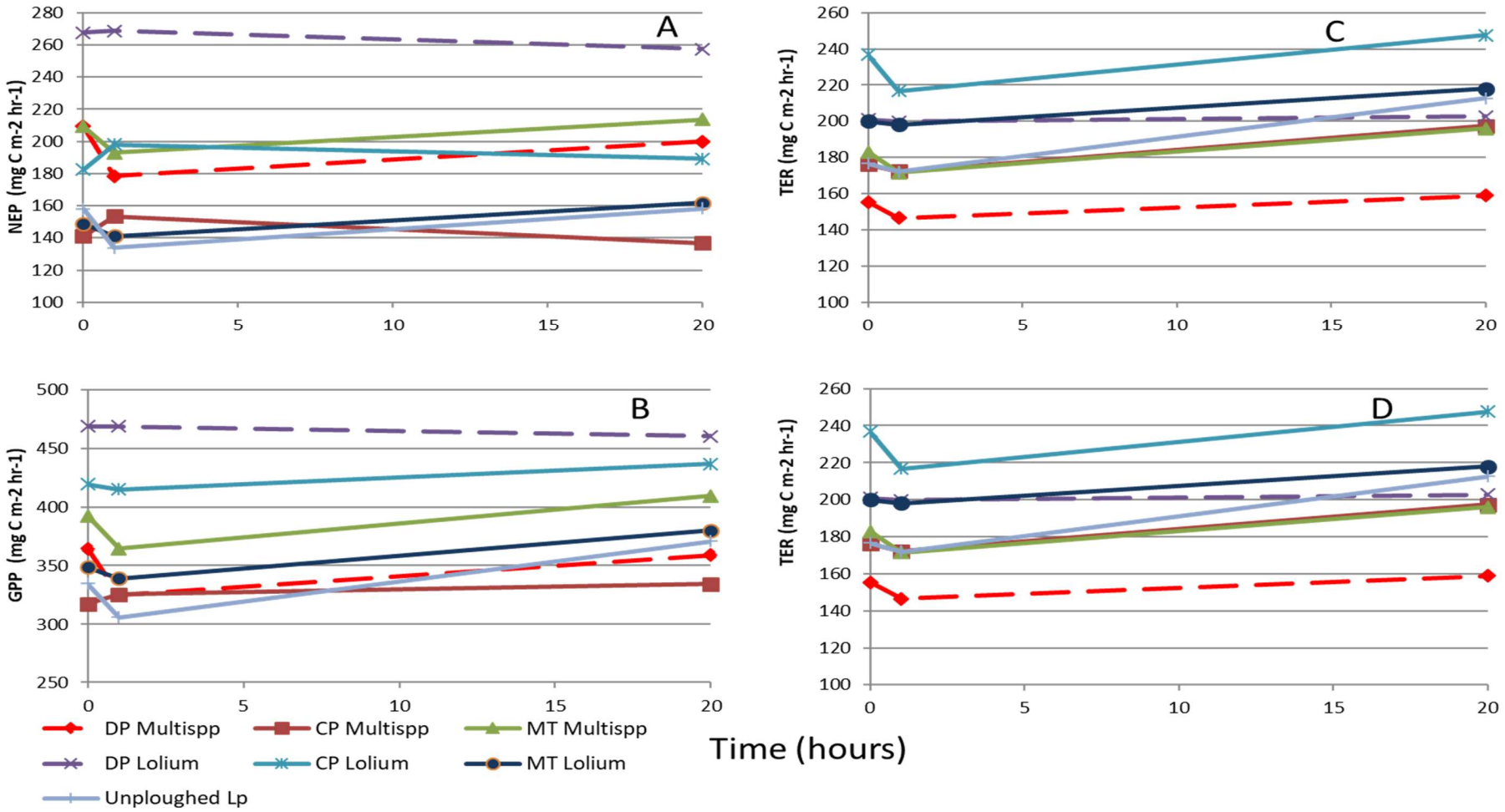

Fig. 8: A) Net Ecosystem Productivity (NEP), B) Total Ecosystem Respiration (TER), C) Gross Primary Productivity (GPP) and D) Soil Respiration for multispecies mixtures and *Lolium perenne* re-established following deep ploughing, conventional ploughing or minimum tillage. Undisturbed *Lolium perenne* lysimeters were used as a control.

Upon introduction of the ^{13}C isotopic tracer, uptake was rapid in herbage with $\delta^{13}C$ substantially increased across of all treatments at one hour post commencement of labelling, due to rapid cycling of photosynthate within the leaves (Figure 9). Incorporation of the ^{13}C tracer into root material was slower, as a substantial portion is metabolised for plant maintenance and no detectable enrichment was detectable in soil organic matter. There was an appreciable enrichment of the ecosystem respired CO_2 with enrichment of between 4.5 and 8.5 ‰. This could be used in conjunction with foliar enrichment to calculate the fraction of TER attributable to foliar autotrophic respiratory component and this was calculated to consume between 13-19% of GPP (Figure 10A). Enrichment of root material continued at a linear rate as labelled photosynthate was translocated to the roots. There was some species variation, as the variability in enrichment of root C in the species mixtures was more than double that for *Lolium perenne*. The difference in ^{13}C between root and soil was used to partition the soil respiratory flux into auto- and heterotrophic gross component fluxes (Figure 10B). There appears to be a consistent pattern in the autotrophic respiration dominates deep ploughed treatments, whilst conventional ploughed/ minimum tillage is equally divided and unploughed grassland is heterotrophic dominated. This is possibly due to a) a lack of microbial biomass and also activity due to substrate limitation in the case of DP treatments, whilst reduced autotrophic respiration is observed in the unploughed lysimeters due to loss of production and indeed comparatively low GPP (which was observed to be 30-40% lower than CP and DP renovated *Lolium* pastures.

4. Future Work

This work will be ongoing and ultimately we hope to trace the C flows through into the labile and recalcitrant C pools. The yield and C sink strength will be continually monitored over the next two years. Based on the high rates of photosynthesis in the renovated treatments, we expect the productivity of the renovated swards to be significantly higher than unrenovated treatments into the future. In conclusion, deep ploughing seems to impact on C balance by qualitatively shifting the balances between GPP, and respiratory loss pathways via changes in nutrient availability and microbial biomass. Our next work will be to investigate the impact on microbial activity, soil enzyme expression and co-limitation impacts of N and P.

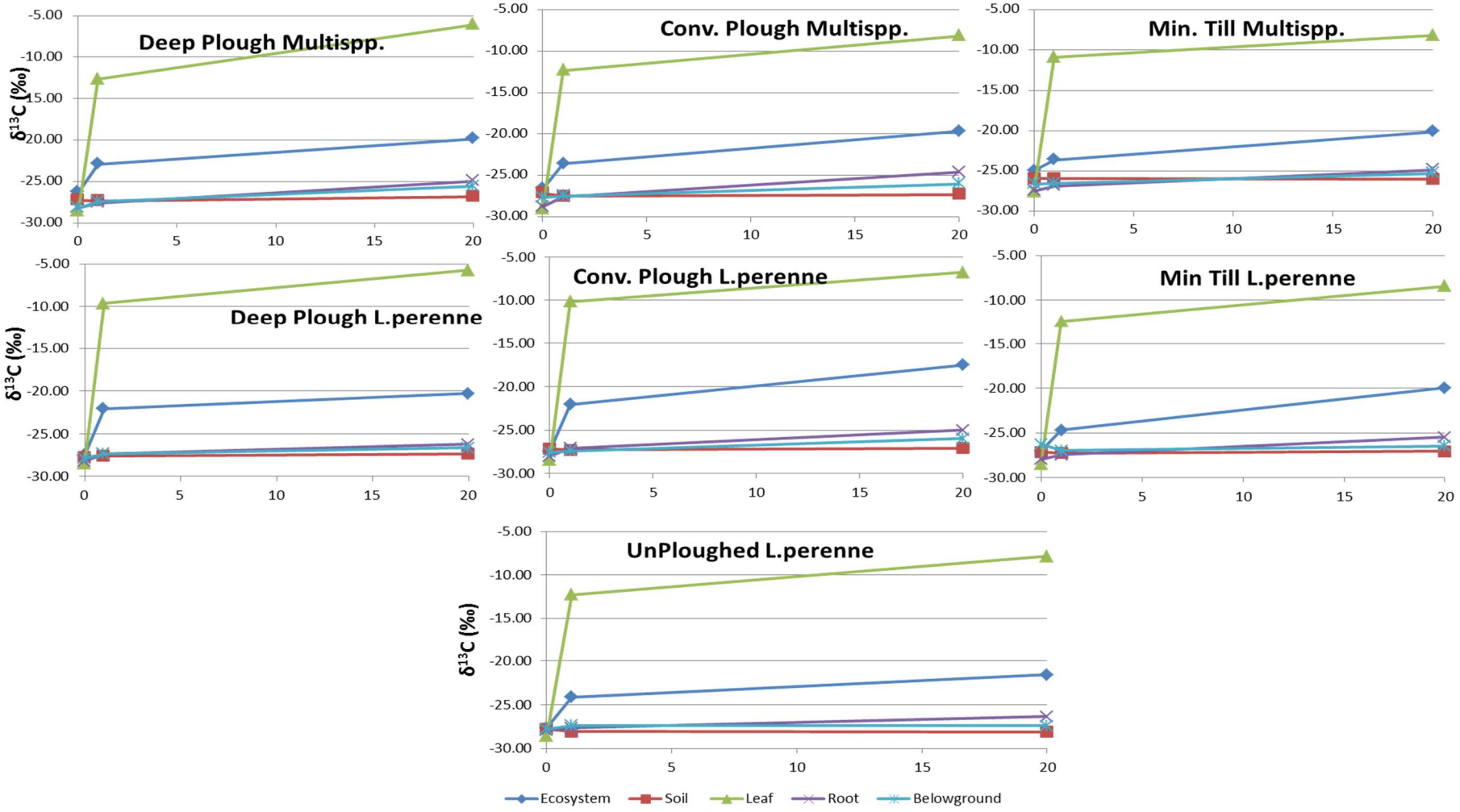

Fig. 9: Temporal pattern of ^{13}C isotope tracer incorporation over 20 hours following the first labelling procedure.

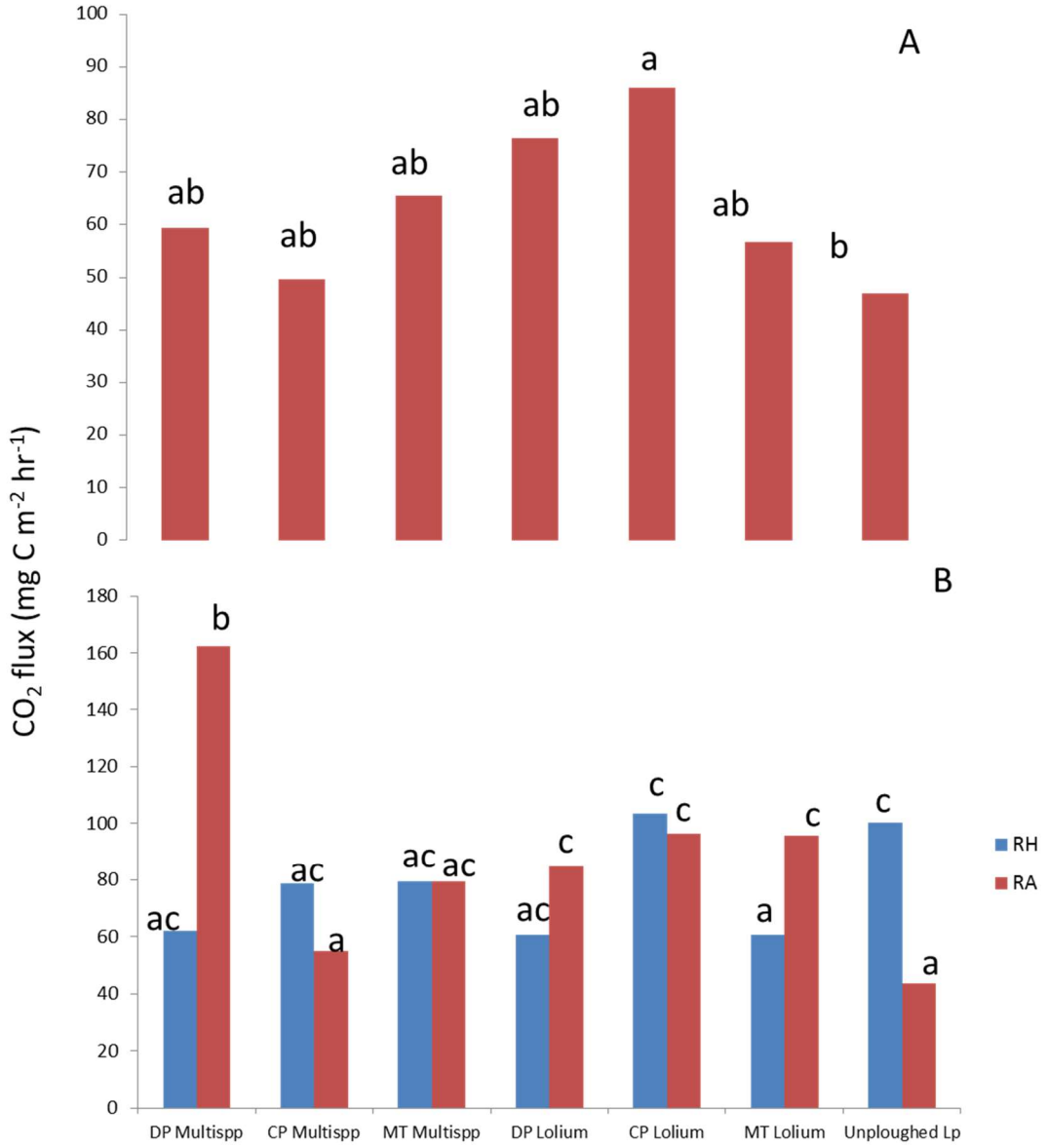

Fig. 10: A) Aboveground (foliar) respiration and b) partitioning of respiration between autotrophic (red) and heterotrophic (blue) component fluxes. Different letters indicate significant differences ($p < 0.05$)

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