GREATSOILS

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Soil Biology and Soil Health Partnership Project 12: Testing soil health and resilience using soil respiration activity

Bussell, J. [1]

[1]The Allerton Project, Game and Wildlife Conservation Trust, Loddington House, Loddington, Leicester LE7 9XE, UK

This is the final report of a 12-month project (Project 12) within the Soil Biology and Soil Health Partnership (AHDB: 91140002) which started in April 2020. The work was funded by a contract for £7000 from AHDB and BBRO.

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1. Abstract

This project is part of a suite of integrated projects (Soil Biology and Soil Health Partnership) specifically aimed at addressing the AHDB and BBRO Soils Programme call - "Management for Soil Biology and Soil Health". This project was one of a number of activities funded through the Innovation Fund, designed to address knowledge gaps that arose over the 5-year duration of the programme (Figure 1).

This work used the existing Soil Biology and Soil Health Partnership no-till field experiment at the Allerton Project, Loddington, to investigate the effects of long term no-till management on functional shifts in the soil microbial community. Within the >7 years direct drilled field, three strips were ploughed to test the impact of ploughing into a previously undisturbed soil. In 2020, after three years of the cultivation trial, samples were taken to measure soil respiration in the plough and direct drill treatments. Functional microbial activity and diversity was assessed using the MicroResp™ method, and functional activity was monitored across the season using in-field soil respiration flux measurements. These were then compared to qualitative genetic diversity measured by DNA analysis, and other measures of soil health taken from soil after harvest of the wheat crop.

CO_2 was found to be higher overall in the direct drilled plots, presumed to be due to greater microbial activity in the less disturbed plots. Soil organic matter was higher in direct drilled plots and had a positive relationship with CO_2 flux and MicroResp™ functional diversity and activity. N_2O was not seen to increase in either the direct drilled or ploughed plots. MicroResp™ assessment showed a shift in the microbial community in the direct drilled plots compared to ploughed plots, with overall greater activity recorded in the direct drilled plots and an ability to metabolise a wider range of substrates suggesting also a greater functional diversity. DNA analysis did not show significant differences in qualitative genetic diversity between plots, and only weak relationships between genetic and functional diversity measured using respiration measurements. This could be due to the lower replicate number (n=6) and high variability within soil samples. The longevity of DNA within soil may also obscure some of the short-term treatment effects.

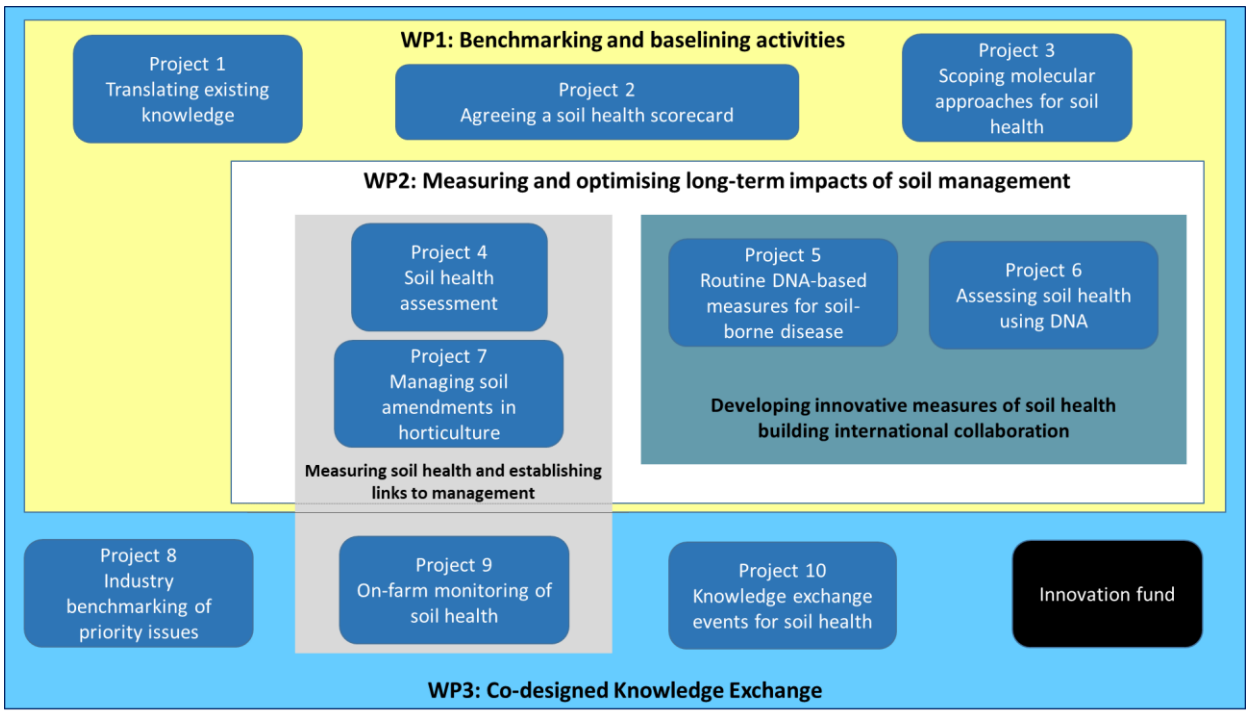

Figure 1. Diagram to show how this project fits into the organisation of the Soil Biology and Soil Health Partnership: Project 12 is one of the projects funded under the “Innovation fund”

2. Introduction

As part of the Soil Biology and Soil Health (SBSH) Partnership a field that had been exclusively no-till for seven years had three strips ploughed in autumn 2017, giving us valuable information on the impact of ploughing a no-till system. Data have been collected on a wide range of soil health indicators including earthworm abundance, soil meso- and microfauna and microbial biomass (Project 4 of the SBSH Partnership). DNA analysis of soil communities can help us estimate the microbial biomass of the soil, the change in the soil community and the diversity of the microbial community. So far in the SBSH Partnership DNA analysis has given us valuable information on the taxa and diversity of bacterial and fungal communities within the soil. However, DNA persists within the soil when soil microorganisms are dead or dormant, so can be slow to identify changes occurring within the soil (Carini et al. 2016), and differences in the physiology of fungi and bacteria can also lead to over or under estimation of abundance when using DNA analysis techniques such as qPCR as an estimation for biomass (Strickland and Rousk 2010). This project used a combination of soil respiration measured *in situ*, alongside MicroResp™, a laboratory-based community-level physiological profile (CLPP) respiration method, to give additional information on the functional changes in the soil community, which can be used to corroborate microbial community shifts seen in DNA analysis.

Soil microorganisms are a crucial part of soil health, performing important roles in nutrient cycling and carbon storage. In soils, N_2O is produced by bacteria through nitrification and denitrification (Firestone and Davidson 1989). Soils become anaerobic quickly when water filled pore spaces (WFPS) are greater than 65% and denitrification becomes the dominant source of N_2O production. Nitrification is a specialised process, while denitrification is relatively ubiquitous, so produces the larger contribution to N_2O production in soils. No-till systems are often associated with greater soil bulk density, causing higher WFPS and anaerobic conditions to occur at lower soil water content (Palm et al., 2014). Conversely, the reduction in pore size distribution in reduced tilled soils has also been linked to lower CO_2 production as anaerobic conditions slow down the mineralisation of soil organic material (Mangalassery et al., 2014). It has also been shown that increased N_2O production in reduced tilled soils is closely linked to increased microbial biomass, as the microbial community benefit from reduced disturbance (Mangalassery et al., 2014). The respiration activity of the microbial community has often been considered in terms of soil health, but with the drive towards net zero greenhouse gas production in agriculture the question of greenhouse gas emissions under different land management has become an important issue to understand.

Functional diversity is often defined as the capacity of the soil community to breakdown a range of different substrates and can be measured using the MicroResp™ system. Functional diversity has been used to look for links between soil management and changes in the soil microbial community. Soil respiration has been used as an indication of soil fertility, with studies finding direct links between

soil microbial respiration and nutrient availability (Burton et al., 2010). Lagomarsino et al. (2007) found soils without nitrogen limitation had higher substrate usage and Zhou et al. (2012) found that growing winter crops increased microbial metabolic diversity, with overall respiration influenced by soil pH and crop biomass. Using this technique alongside other measures taken in the SBSH Partnership we can explore links between indicators of soil health and improvements in soil microbial metabolic activity and diversity, which are both key for organic matter decomposition, storage, and mineralisation of nutrients.

3. Materials and methods

3.1. Experimental set up

The experimental site was established in 2017 on a field at the Allerton Project - a 300 hectare mixed arable and livestock Research, Demonstration and Education Farm (Game & Wildlife Conservation Trust), in Loddington, Leicestershire, UK (N 052°36′53″ W 00°50′31″; 186 m above sea level). Soils are predominantly a heavy clay loam, from the Denchworth series: texture 45% clay, 35% silt, and 20% sand. The field had previously had no cultivation for over 7 years. Six plots were established, each 9 metres wide, running the full length of the field with a 24-metre gap from the headlands (Figure 2). Plots were randomly assigned as plough or direct drill. Plough plots were mouldboard ploughed to a depth of 25 cm, then disked to a depth of 10 cm (Cäderstad carrier) twice every Autumn, while direct drill plots received only a straw rake before drilling. Plots were drilled (Eco M, Dale drills) with winter wheat in 2017, oats in 2018 and spring wheat in 2019. Standard farm practice was used for fertilizer and chemistry application, and this was consistent across all plots. Sampling for this project took place in 2020 (during the spring wheat cropping season).

Figure 2: 9-metre wide plough and direct drill plots running across the field, with Gasmet gas analyser sitting on a ring measuring field greenhouse gas flux.

3.2. MicroResp™

Soil samples were taken for MicroResp™ (James Hutton Institute) at four time intervals during the 2020 season (April, May, July and September). Samples were taken from 5 points within each plot using a trowel to a depth of 15 cm. These samples from each replicate plot were then mixed and sieved to 2 mm. To allow a comparison between MicroResp™ and soil DNA analysis, the thoroughly mixed soil samples were then sub-sampled for both MicroResp™ and DNA analysis (with DNA analyses performed by FERA as part of SBSH Partnership Project 5&6 activities). A further subsample of this soil was also taken for soil organic matter analysis in September, using the loss on ignition technique: samples were dried at 105°C for 24 hours, then weighed before being placed in a muffle furnace at 550°C for 8 hours and weighed again to determine the weight loss, which was calculated as the % organic material within the sample.

Soil samples for MicroResp™ analysis were stored in the fridge at 5°C prior to analysis, for no more than 4 days, then incubated for 3 days at 21°C in a sealed container with wet paper towels and soda lime as per the MicroResp™ protocol. A mixture of organic acids, amino acids and sugars commonly found in soils were used as the substrates (Table 1) and the standard MicroResp™ protocol was used to adjust these to soil water content and add them to soil. CO_2 production was then recorded giving a measure of respiration through a colour change reaction on a microplate reader at 570 nm (EMAX plus, Molecular Devices) after 6 hours (Campbell et al. 2003). The September sampling was done after harvesting the Spring wheat crop in 2020, alongside all other measurements taken on the plots as part of the SBSH Partnership monitoring (Project 4).

Table 1: The substrates used in the MicroResp™ analysis.

1	trehalose
2	citric acid
3	malic acid
4	3,4-Dihydroxybenzoic acid
5	oxalic acid
6	arabinose
7	aminobutaric acid
8	fructose
9	L-arginine
10	N-acetyl glucosamine
11	L-alanine
12	glucose
13	Aspartic acid
14	galactose
15	water

3.3. Field gas flux

A DX4040 (Gasmet technologies LTD) Fourier-transform infrared (FT-IR) spectrometer, combined with a 20 cm soil respiration chamber (Li-Cor biosciences) was used to measure nitrous oxide (N_2O) and carbon dioxide (CO_2) gas flux directly from the soil in the field (Figure 2). A plastic collar was placed in the soil, with 15 cm buried in the soil to prevent leaking. The soil respiration chamber was placed tightly onto the collar to make an airtight seal forming a closed system. The air was circulated round the analyser for 10 mins recording the increase in gas concentration and from this the gas flux was calculated. Soil gas flux was measured at five separate intervals across the 2020 season: April, June, July, September and November. As N_2O has 298 times more global warming potential than CO_2 (Solomon, 2007) the number recorded from the field is multiplied by 298 and reported as CO_2 equivalent (eCO_2) to allow for easier comparison of the global warming potential of the two gas fluxes.

Alongside the gas flux measurements soil moisture was measured to a depth of 20 cm using a moisture meter (Field Scout, TDR 100) and soil temperature was measured to a depth of 15 cm using a digital thermometer (Fisherbrand, Tracable). Five measurements were taken per plot and averaged for both moisture and temperature. As no difference was seen between treatments these results were averaged across all plots to demonstrate differences across sampling periods (Figure 8).

3.4. Data analysis

For the MicroResp™ system, the average metabolic response (AMR) was calculated as the average total response (μg CO_2-C g^{-1} soil h^{-1}) of soil to all substrates added, giving a measure of microbial activity. Community metabolic diversity (CMD) was calculated by summing the positive responses to substrate additions. It is a simple measure of ability of the soil microbial community to effectively metabolise the substrates given, so is a measure of functional diversity. For field CO_2 flux, N_2O flux and MicroResp™ AMR and CMD results, a repeated measures ANOVA was used to compare between treatments. To further visualise the MicroResp™ results a principal components analysis (PCA) was used to reduce the dimensionality of the data down from 15 substrates to two PC axis, containing 70.52 % and 16.22 % of the data variability respectively.

Data on bacterial and fungal DNA diversity were provided from FERA as a Shannon diversity index. MicroResp™ AMR and CMD results, and gas flux results were all averaged over the sampling time periods to give one measurement per plot for regression analysis. Linear regressions were used to compare between field CO_2 flux, N_2O flux, MicroResp™ AMR, CMD, and bacterial and fungal DNA diversity measured in SBSH Partnership Project 6, and soil health properties measured as part of SBSH Partnership Project 4.

4. Results

4.1. MicroResp™

Repeated measures analysis of the MicroResp™ AMR and CMD data showed significant (P=0.007; P=0.006 respectively) treatment effects, with direct drilled plots having greater AMR and CMD responses. There were large variations between the sampled dates (Figures 3 and 4) with the largest differences between treatments seen in April, and the largest overall response seen in July. Although time factor was just out of the significant range (P=0.053) for AMR, it was significant for CMD (P=0.016). There was no treatment * time interaction for either AMR or CMD. Principal Component analysis of the MicroResp™ results showed a divergence between direct drill and plough plots mostly on the PC1 axis, which contained 70.52% of the overall data variability. The substrates that loaded highest on this axis were fructose and malic acid. The direct drilled plots had more variation between them than the plough plots, and this variation was seen mostly on PC2 (16.22% variation), which was most highly influenced by the substrates aminobutaric acid, and glucose, with a slight negative influence from malic acid (Figure 5).

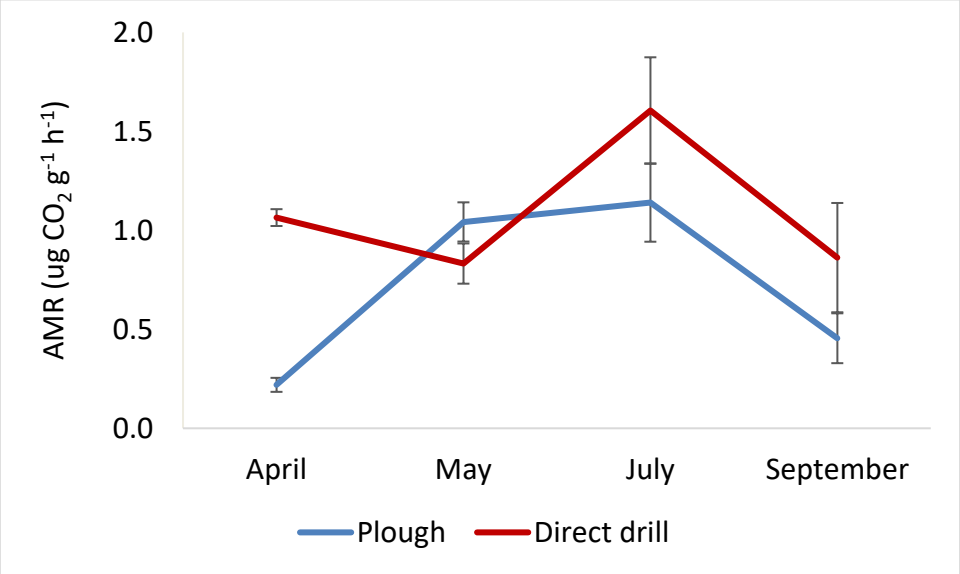

Figure 3: Average metabolic response (AMR) from MicroResp™ analysis over time. Error bars show 1 standard error from mean.

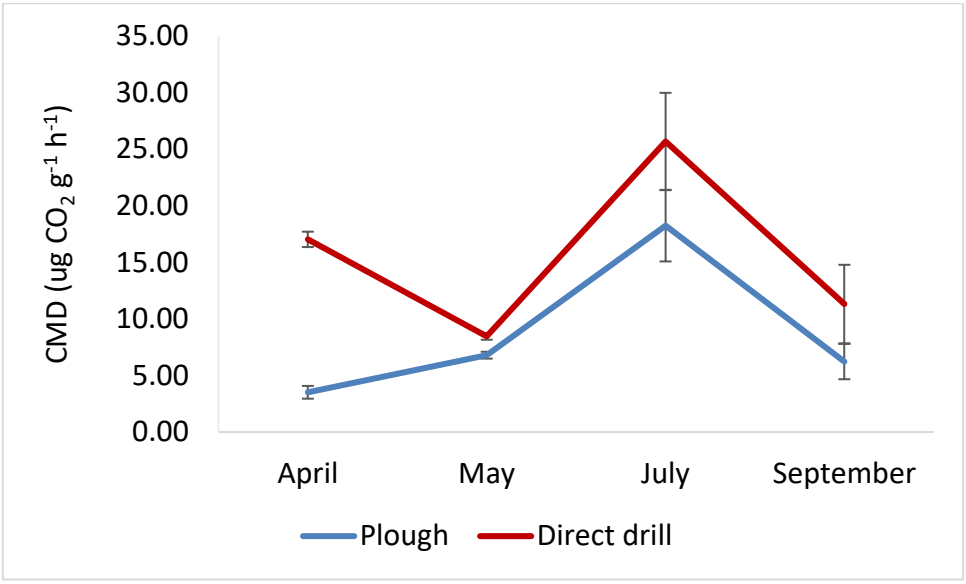

Figure 4: Community metabolic diversity (CMD) from MicroResp™ analysis over time. Error bars show 1 standard error from mean.

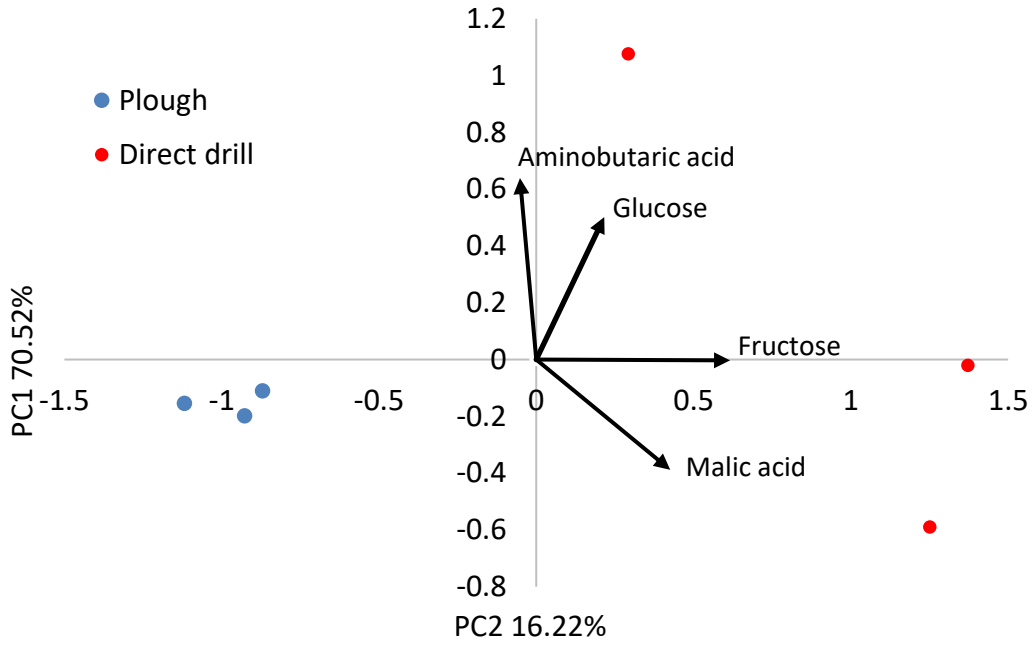

Figure 5: PC1 and 2 from PCA of MicroResp™ data from all 15 substrates. Black arrows show PC loadings of the 4 most influential substrates.

4.2. Field gas flux

Field CO_2 flux showed a significant treatment response (P=0.028), with direct drilled plots having higher CO_2 flux than ploughed plots (Figure 6). There was no significant time effect (P=0.77) or treatment * time interaction (P=0.33) despite large variation between sampling points. Field N_2O flux showed no significant treatment, time, or interaction effects (P=0.128; P=0.272; P=0.367

respectively), due to the overall low values recorded across the sampling points (Figure 7). The largest peak was seen in April, and this may be due to the wettest soils seen at this sampling time (Figure 8). Fertilizer was spread on the field at the end of April, after the April measurement was taken, so the flush of N_2O release in April was not due to fertilizer application.

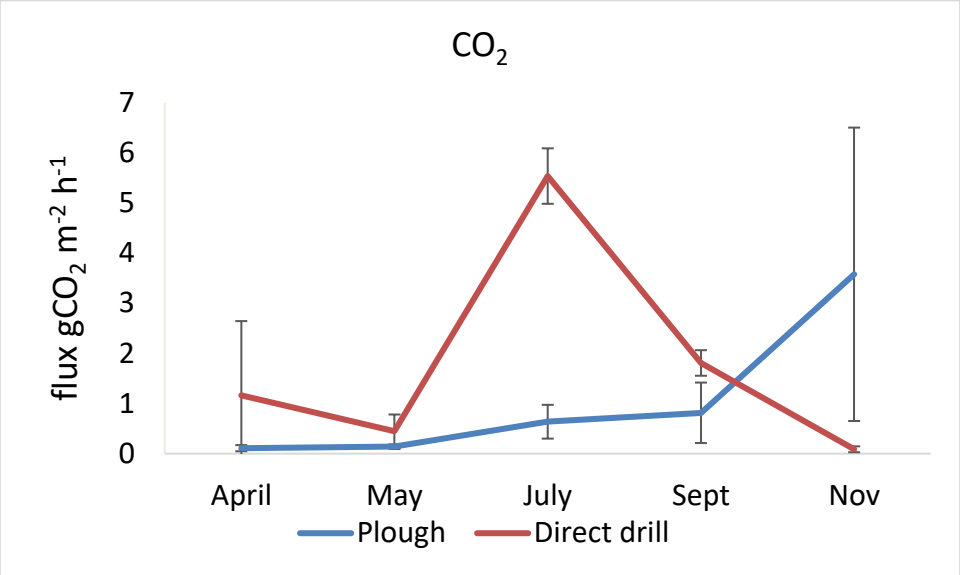

Figure 6: CO_2 flux from field measurements over time. Error bars show 1 standard error from mean.

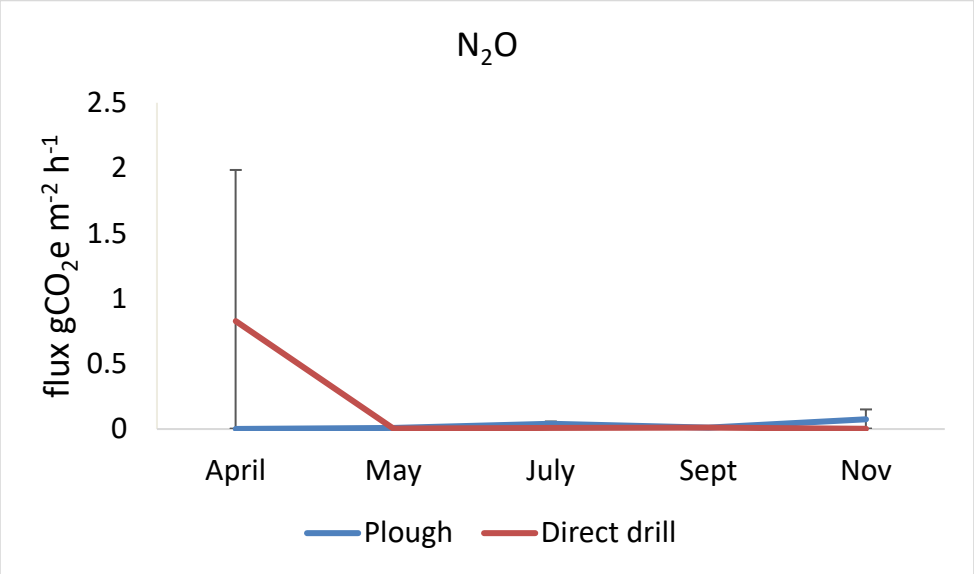

Figure 7: N_2O flux from field measurements over time. Error bars show 1 standard error from mean.

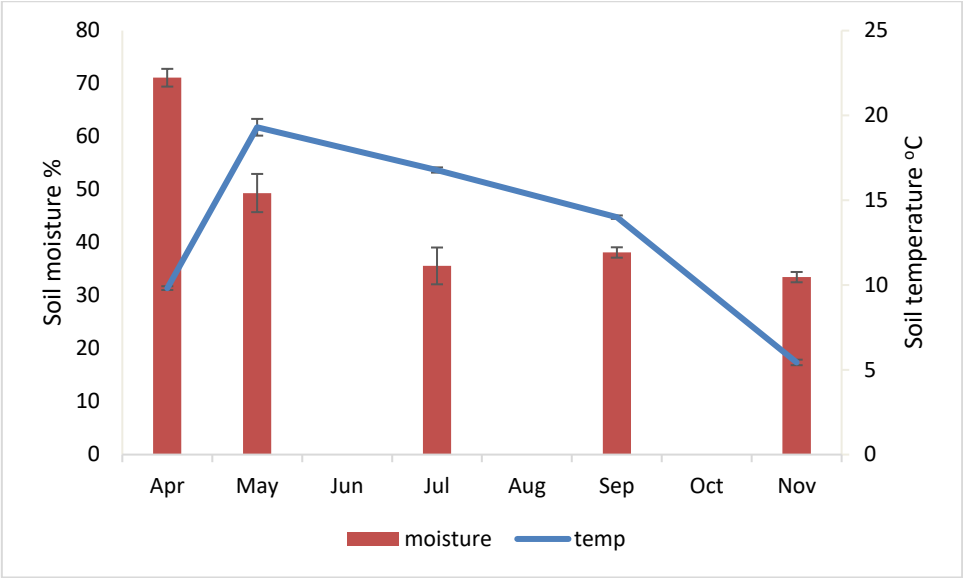

Figure 8: Soil moisture and temperature data taken at the time of soil flux measurements. Error bars show 1 standard error from mean.

4.3. Regression analysis

Regression analysis compared the Shannon diversity index calculated from fungal and bacterial DNA sampled as part of SBSH Partnership Project 6, to the MicroResp™ CMD and AMR; and the field CO_2 and N_2O flux collected in this project, averaged over the sampling times to give one result per plot. Very few significant regressions were seen mostly due to overall variability in the data and the low number of data points (n=6). As part of the focus of this work was to investigate potential relationships between these parameters some graphs showing non-significant trends have been included in the results to show potential areas for further investigation, but these data must be dealt with cautiously.

A significant relationship was seen between MicroResp™ CMD and field CO_2 flux (P=0.005, r^2 =0.8915, Figure 9), with greater CMD and higher CO_2 flux seen in direct drilled plots. A significant relationship was also seen between CMD and N_2O field flux (P=0.012, r^2 =0.8284, Figure 10), with lower N_2O production seen in the direct drilled plots. A similar significant relationship was also seen between AMR and field CO_2 flux (P=0.023, r^2 =0.764, Figure 11), but the relationship between AMR and N_2O flux was non-significant (P=0.063, R^2=0.621)

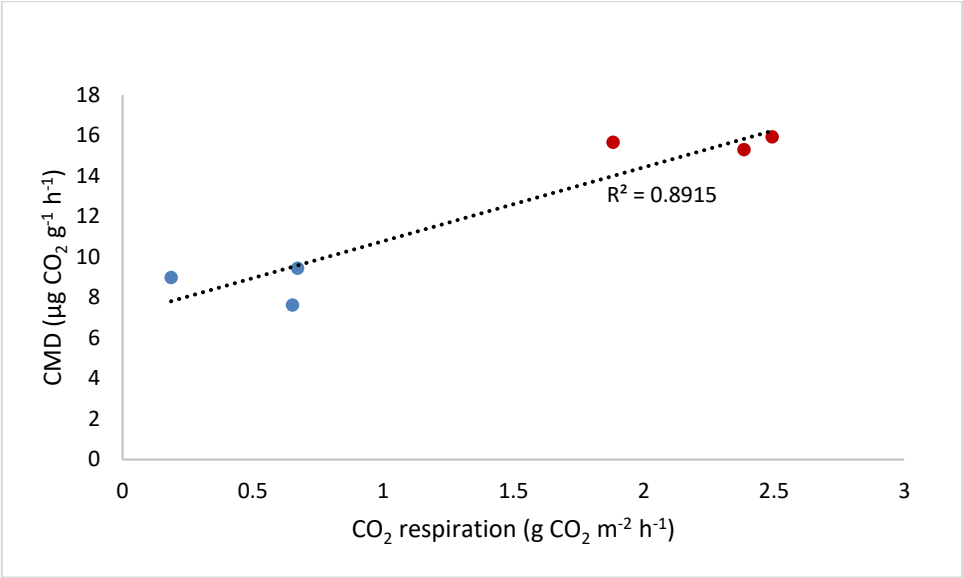

Figure 9: Linear regression of MicroResp™ CMD vs. CO_2 field flux. Red dots represent direct drilled plots, blue dots represent plough plots.

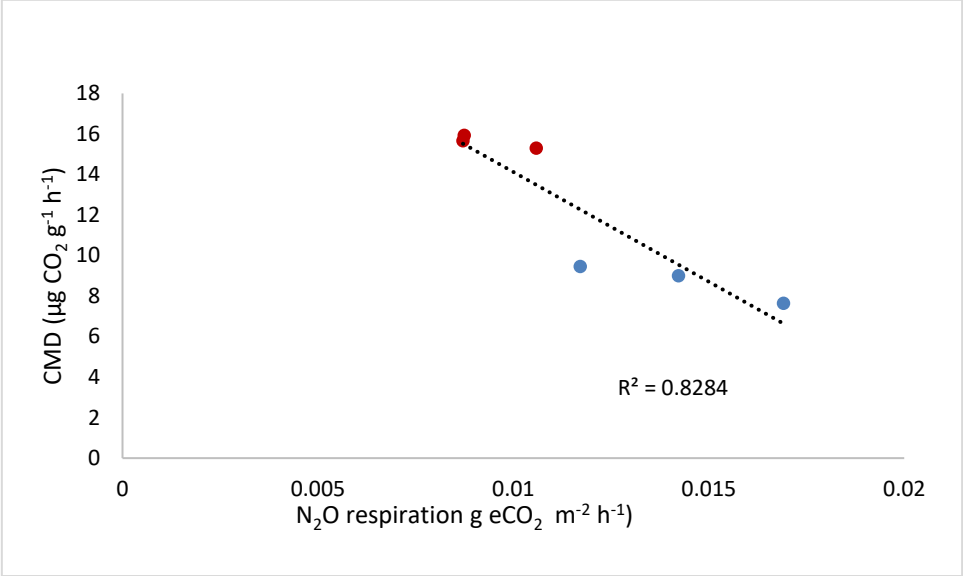

Figure 10: Linear regression of MicroResp™ CMD vs. N_2O field flux. Red dots represent direct drilled plots, blue dots represent plough plots.

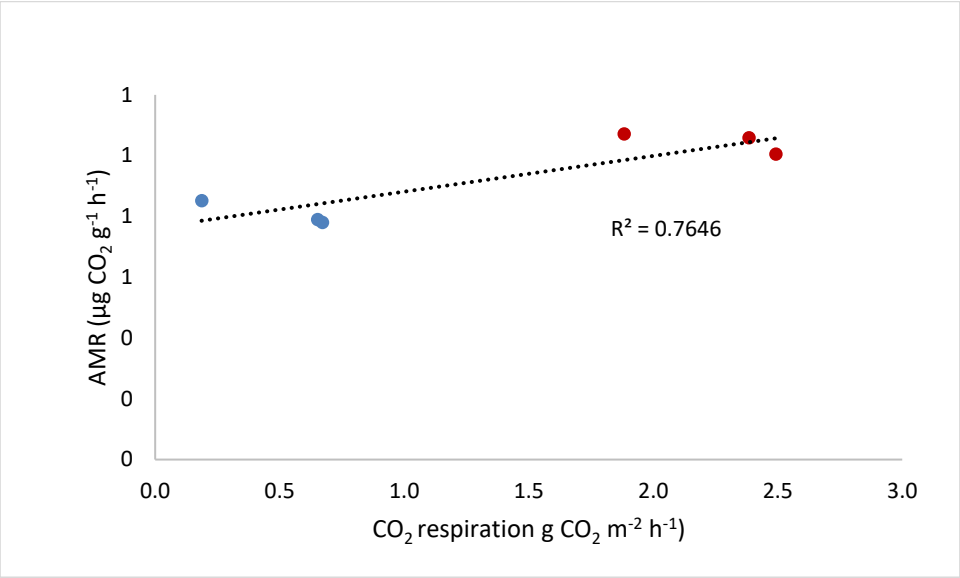

Figure 11: Linear regression of MicroResp™ AMD vs. CO_2 field flux. Red dots represent direct drilled plots, blue dots represent plough plots.

DNA fungal diversity showed a non-significant relationship (P=0.153, R^2=0.437, Figure 12) with field CO_2 flux, and no significant relationship with N_2O flux (P=0.262, R^2=0.298). DNA fungal diversity had no significant relationship with the MicroResp™ index AMR or CMD (P=0.508, R^2=0.279; P=0.281, R^2=0.116 respectively). DNA bacterial diversity showed no relationship with CO_2 or N_2O flux or with the diversity index CMD calculated from MicroResp™ (P=0.471, R^2=0.137; P=0.327, R^2=0.237; P=0.228 R^2=0.336 respectively). There was a non-significant (P=0.082, R^2=0.572, Figure 13) negative relationship between DNA bacterial diversity and MicroResp™ activity index AMR, with direct drilled plots having the lowest bacterial diversity and highest AMR.

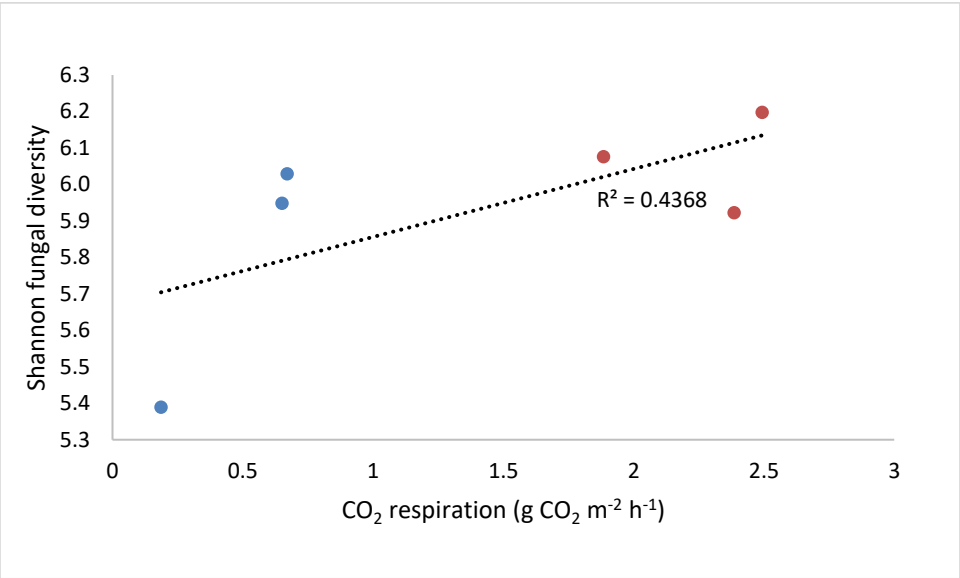

Figure 12: Linear regression of DNA fungal diversity vs. CO_2 field flux. Red dots represent direct drilled plots, blue dots represent plough plots.

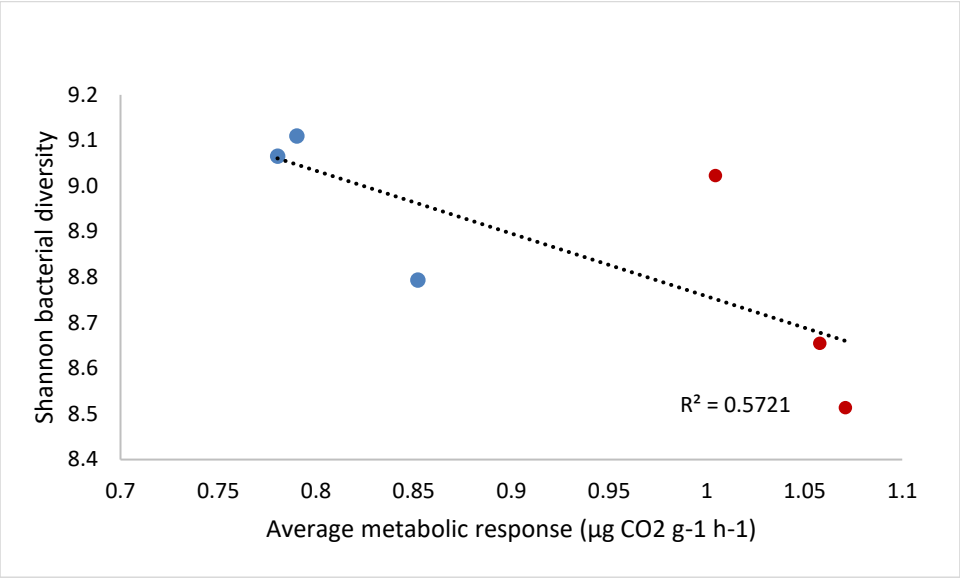

Figure 13: Linear regression of DNA bacterial diversity vs. MicroResp™ AMR. Red dots represent direct drilled plots, blue dots represent plough plots.

Soil organic matter was measured using the loss on ignition (LOI) method on subsamples from the soil collected for MicroResp™ and DNA analysis in September. A significant positive relationship was seen between soil organic matter and MicroResp™ CMD and AMR results (P=0.003, r^2=0.9088, Figure 14; P=0.015, r^2=0.807, Figure 15 respectively), with higher CMD, AMR and SOM in direct drilled plots.

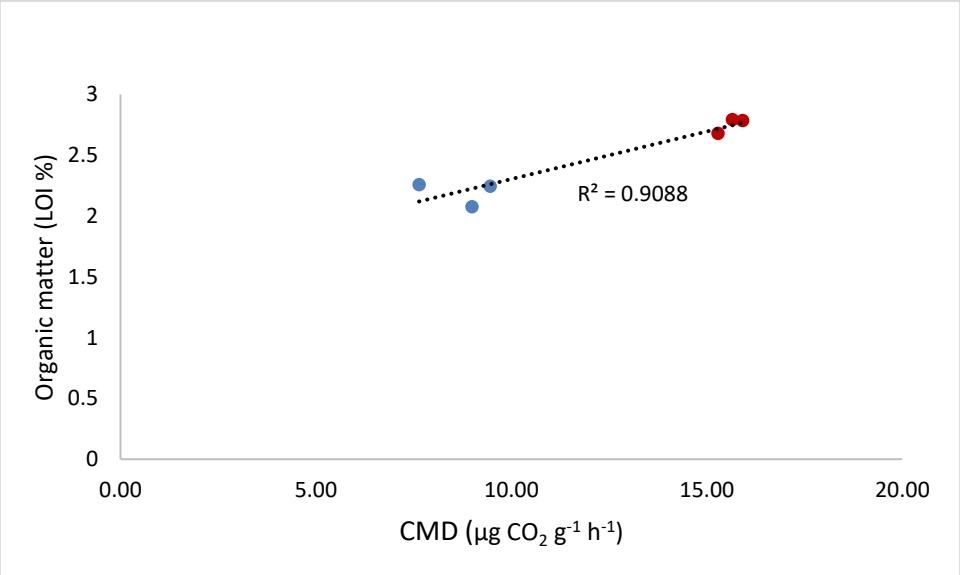

Figure 14: Linear regression of organic matter vs MicroResp™ CMD. Red dots represent direct drilled plots, blue dots represent plough plots.

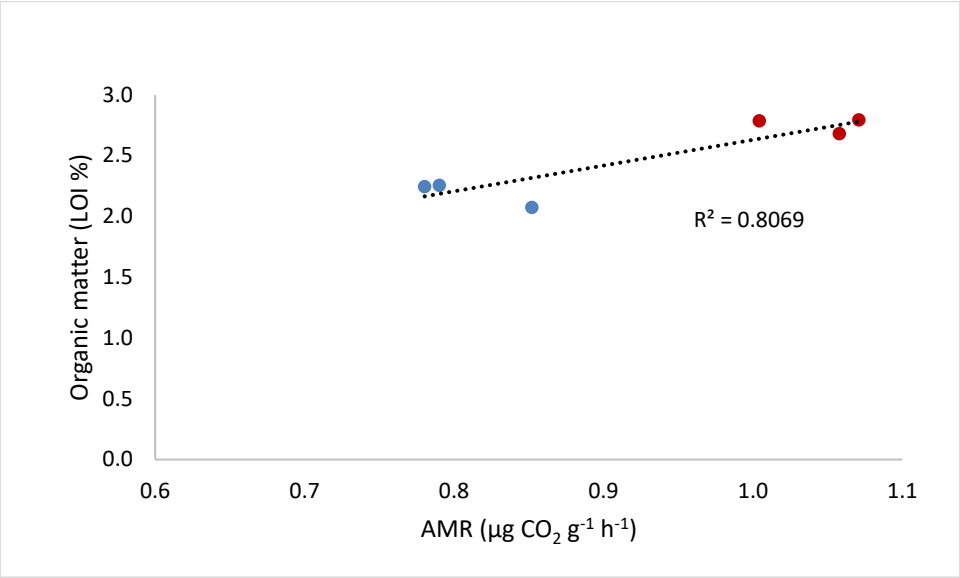

Figure 15: Linear regression of organic matter vs MicroResp™ AMR. Red dots represent direct drilled plots, blue dots represent plough plots.

A significant relationship was also found between organic matter and both CO_2 and N_2O soil flux (P=0.002, r^2=0.920, Figure 16; P=0.041, r^2=0.687 Figure 17 respectively). CO_2 flux regressed positively with SOM, while N_2O flux regressed negatively with SOM. SOM was significantly higher in direct drilled plots (P=0.002, mean 2.3 % +/- 0.1 plough; 2.8 % +/- 0.1 direct drill).

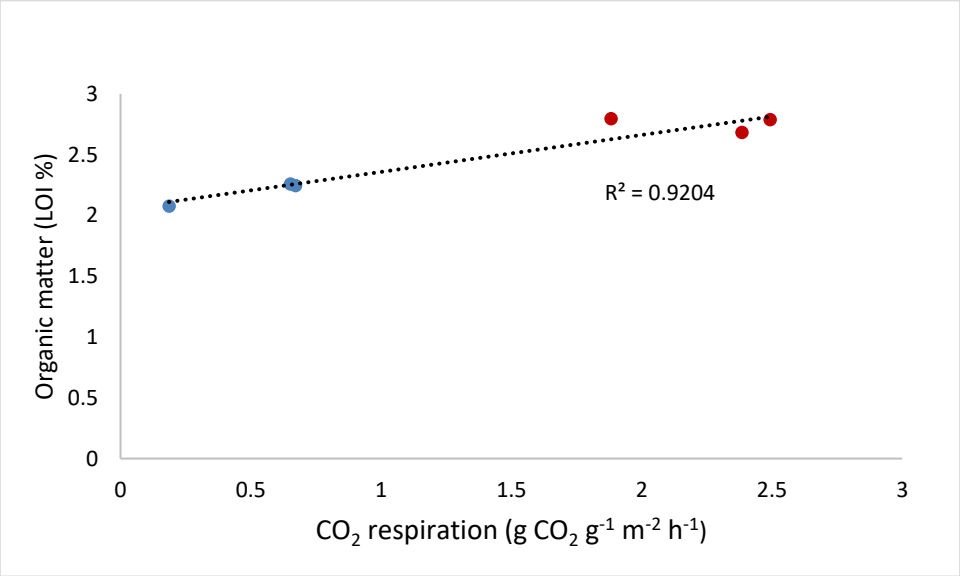

Figure 16: Linear regression of organic matter vs soil CO_2 flux. Red dots represent direct drilled plots, blue dots represent plough plots.

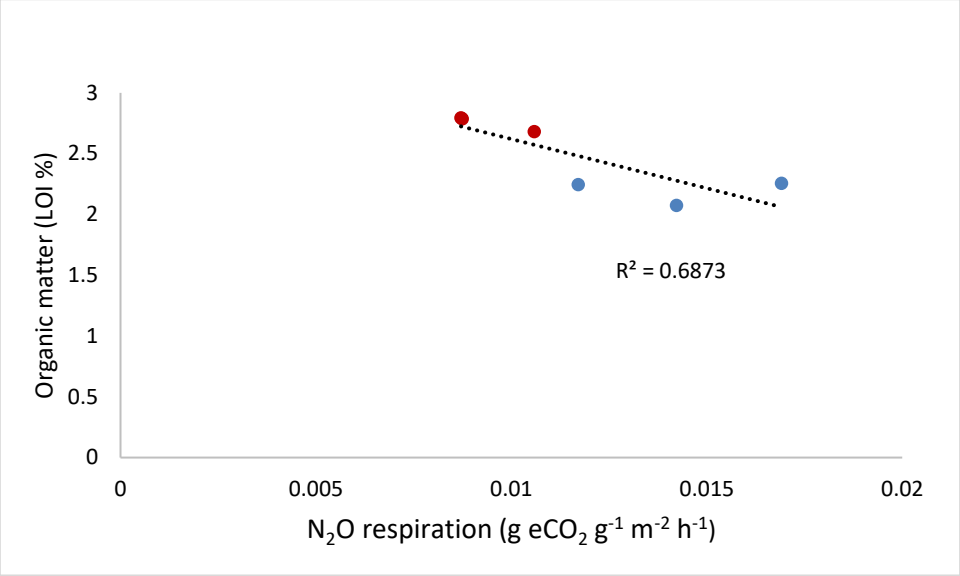

Figure 17: Linear regression of organic matter vs soil N_2O flux. Red dots represent direct drilled plots, blue dots represent plough plots.

5. Discussion

As a measure of soil activity, respiration was measured using both *in-situ* field respiration measurements of CO_2 and N_2O, alongside taking fresh samples from the field for substrate induced respiration, using the MicroResp™ system. Community metabolic diversity (CMD) and average metabolic response (AMR) were both calculated from the MicroResp™ system giving a measure of microbial diversity and activity. The MicroResp™ system showed significant differences between the AMR and CMD of the plough and direct drilled plots. Overall, the microbial community within the direct drilled plots were able to breakdown a larger proportion of the 15 different substrates provided, showing a more functionally active and diverse microbial community. As the microbial community is responsible for nutrient cycling in soils, diversity can influence nutrient availability. Diversity can also confer resilience onto a system, as more than one pathway can be available to do many soil functions, allowing others to take over if one becomes disrupted and diverse microbial communities have been shown to be more efficient at resource utilization (Mäder et al 2002).

To further investigate the responses of the soils to the substrates added in the MicroResp™ system, principal component analysis (PCA) was used. PCA showed separation between direct drill and ploughed plots was mostly along PC1, which was most heavily influenced by the sugars glucose and fructose and the organic acid, malic acid. The direct drill plots had the largest variation between them, also separating on PC2, which was influenced negatively by malic acid, and positively by glucose and aminobutaric acid. Glucose and fructose are simple sugars, deposited as part of root exudates and quickly utilized by microbial systems (Paterson et al, 2007). The treatment separation on PC1 due to differences in response to these simple sugars may signal an overall greater microbial

biomass in the direct drilled plots rather than a more functionally specialised microbial community, as most microorganisms would be able to break down these substrates. Microbial biomass was measured as part of SBSH Partnership Project 4 and was on average 27% higher in direct drilled plots, but this difference was not significant.

Malic acid is an organic acid, also produced by plants and excreted in rhizodeposition. It is often produced in response to phosphate limitation helping plants acquire phosphate by chelating the ions that bind to form insoluble salts with phosphate (Paterson et al., 2007). Malic acid has also been identified as a signalling molecule for beneficial bacteria such as *Bacillus subtilis*, which can confer some protection from pathogenic bacteria in plants (Rudrappa et al., 2008). Aminobutaric acid is another signalling molecule, used by plants to stimulate the production of defensive compounds such as phenolic acids in response to biotic and abiotic stress, including drought and pathogens (Ramesh et al. 2015). The large diversity of substrates that the microbial communities in the direct drill plots responded to suggest this community is able to respond to a greater array of root exudates.

The field respiration showed significantly higher CO_2 emissions from the direct drilled plots, with a large amount of seasonal variability. In contrast to findings here, previous research has shown direct drill plots to have lower CO_2 flux than plough plots, due to the lack of air-filled pore space slowing down the mineralisation of soil organic material (Mangalassery et al. 2014). This increase in CO_2 flux could suggest that the direct drilled plots have a larger, more active microbial community than the plough plots. N_2O readings were low across all timepoints measured but were within the range measured in other UK wheat fields (Drewer et al. 2012). N_2O is often hypothesised to increase in direct drilled plots also due to the lack of air-filled pore spaces increasing activity of denitrifying bacteria, but in this study N_2O emissions did not significantly differ between the two plots and were consistently low throughout the year with a slight non-significant increase in direct drill plots for the wettest sampling point, April. As the direct drilled plots had been in place for >10 years by the end of the experiment a gradual improvement in soil structure has had time to take place, so initial problems associated with compaction and increased water filled pore space, causing a spike in denitrifying bacteria N_2O production were not apparent during this study.

Regression between field respiration and CMD recorded using the MicroResp™ system showed a significant positive relationship with CO_2 and a significant negative relationship with N_2O. AMR also showed a significant positive relationship with soil CO_2 flux suggesting both higher metabolic activity and diversity were driving the increased field respiration. The direct drilled plots had the highest CO_2 production and lowest N_2O production suggesting that the shift in the microbial community under direct drill treatment had caused an overall increase in microbial activity. The decreased N_2O production in direct drilled plots may be due to slight improvements in soil structure over time,

decreasing the water filled pore spaces and so reducing the denitrifying activity which produces the largest contribution to N_2O fluxes.

As part of the SBSH Partnership Project 6, 'Assessing soil health using DNA', samples were taken from the ploughed and direct drilled plots for DNA analysis. For each treatment, these samples were mixed and then split so the same sample was used for functional diversity analysis using MicroResp™, and for DNA diversity analysis enabling comparisons of the two methods for profiling changes in the microbial community. Shannon diversity indexes were generated from the DNA analysis and comparisons of the two treatments showed no significant differences in DNA diversity. Regressions between the DNA diversity and soil CO_2 flux showed a non-significant trend towards increased fungal diversity with an increase in carbon dioxide flux. Microbial communities have been seen to increase in less disturbed soils, with increases in microbial biomass, and a shift towards more fungal dominated communities found in non-tilled soils (Helgason et al. 2010). Fungi in particular are expected to benefit from direct drilling as large hyphal networks receive proportionally more damage from physical soil disturbance (Sun et al. 2020). Although non-significant, greater fungal diversity and larger CO_2 respiration were both recorded in direct drilled plots, this result may be worth further investigation to see if there is an overall increase in microbial community activity, particularly the fungal community within these plots. Similarly, a non-significant trend between Shannon bacteria diversity from DNA analysis and AMR from MicroResp™ metabolic activity index may be worth further investigation as lower bacterial diversity, but greater AMR were recorded in direct drilled plots.

As there are non-significant indications of opposing shifts in diversity of fungi and bacteria between these two treatments it would be interesting to investigate the fungi:bacteria (f:b) ratio further in these plots, to see if the microbial community has also changed in f:b composition. Direct drilling would be expected to benefit the fungal community (Kabir, 2005; Säle et al., 2015). Decomposition of organic matter in soil can be via bacteria or fungal decomposition, research suggests that changes in the f:b ratio can alter the speed and chemical composition of organic matter breakdown, with implications for plant nutrient availability and soil carbon storage (De Vries et al. 2016; Malik et al. 2016).

As part of SBSH Partnership Project 4 many soil health parameters were measured on the plots after harvest in 2020. Soil organic matter (SOM) measured using loss on ignition showed a significant decrease of 0.5 % in ploughed plots. As ploughing oxygenates the soil and allows faster mineralisation of SOM (Cooper et al., 2021), this represents the organic matter lost after 3 years of ploughing a previously no-till field. SOM is built up over time, and microorganisms break down the organic matter and help to further stabilise it within the soil though the production of stable soil aggregates and recalcitrant humic compounds (Stenberg et al., 2000; Cooper et al., 2021). This

creates a positive feedback loop with more SOM creating a larger more active microbial population, which is protected in undisturbed soil. A highly significant (p=0.002) positive relationship was seen both between SOM and microbial activity measured as both field CO_2 flux and the MicroResp™ indices CMD and AMR showing the importance of SOM in microbial activity and diversity. A significant negative relationship was also seen with field N_2O flux which may be a sign of improved soil structure with increased SOM increasing aeration in the soil and reducing the conditions for N_2O production (Franzluebbers 2002).

It is clear from the regression analysis that there is a large amount of variation between the plots, explaining why simple two-way statistical tests have not always shown differences in DNA diversity, or N_2O respiration. This is a common problem for soil analysis, as soil is such a heterogeneous environment; the three replicates used in this study may not be enough to show the small shifts seen within the soil community between these two treatments and also too few to give significant regression relationships. A further complication of soil analysis is the persistence of DNA from dead microorganisms which can persist in the soil for years, obscuring short term treatment effects (Carini et al., 2020; 2016). This could explain the limited relationships seen between DNA analysis, which may show longer term trends in the microbial community, and the MicroResp™ analysis which shows only the response from the living fraction of the microbial community, so may be more sensitive to short term changes due to treatments. Despite the temporal and spatial heterogeneity of the plots there were overall trends showing greater CMD, AMR and field CO_2 flux in direct drilled plots, suggesting a more active and diverse microbial population.

6. References

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