

Please see here below our response to the comments provided by Prof. Schipper (Referee).

Review of: Long-term nutrient fertilization and the carbon balance of permanent grass-land: any evidence for sustainable intensification? D. A. Fornara et al.

This paper examines changes in soil C and N in cut pasture systems following application of different pig and cow manures. Control and NPK fertiliser treatments were included. Soil samples were collected to 15 cm. There are few long-term trials of this nature and they can provide very valuable information. Like many of these trials, it is not likely that they were primarily established to determine changes in total C stocks and so there can be inevitable shortcomings. Here the relatively shallow sampling and few bulk density measurements might be criticised. However, I believe it is better to make the best of the rather unique data that is available for interpretation. Very interestingly, this study finds increases in soil C and N over 43 years for all treatments including the control (no fertiliser treatment) and the accumulation rates are substantial, as great as 0.86 Mg/ha/y. Establishment of a new carbon stock is reasonably well accepted when there is a major change in land use and management.

We really appreciate the very knowledgeable comments provided by Prof. Schipper, which have significantly contributed to improve our manuscript. We agree with the view that long-term grassland experiments are extremely important to understand how the biogeochemistry of these managed ecosystems may be affected by human activities. We also recognize that the application of different management treatments and the addition of more frequent measurements including soil bulk density and other parameters at deeper soil layers might have reduced variability and clarified key trends within our dataset. Nonetheless we would like to highlight the importance of the unique dataset associated with this Long-Term Slurry Experiment, which together with other long-term experiments around the world could contribute to a better mechanistic understanding of how carbon can be lost or gained in permanent grassland soils under intensive management.

The major questions in my mind are: Why the establishment to a new C stock equilibrium is taking so long particularly in the control treatment (still gaining 0.35 Mg/ha/y), which presumably has been under pasture for some time?

Land use previous to the establishment of the Long-Term Slurry Experiment is not known for certain, however, the absence of any specific information suggests that land use was mainly pasture. An important point could be that there had been a 'disturbance' event in 1969 when the pasture was ploughed and reseeded with ryegrass and then the slurry experiment started the following year in 1970. We don't know whether this disturbance event had created the conditions for accumulating more C in the following years. Our evidence is that C has been accumulating even in recent years after few decades since the disturbance event. We have now added this info on page 3, lines 7-9: "The Long-Term Slurry experiment (LTS) was established in 1970 on a pre-existing sward of perennial ryegrass, which was previously established in 1969 after a ploughing and reseeded event".

One of the reasons we think control plots can still show significant rates of C accrual (equal to 0.35 Mg C /ha/y) is due to higher root C pools compared to fertilized plots. High root C pools may provide significant amount of C to soils either via root exudation mechanisms or root decomposition. We have been now planning to measure root decomposition rates in these plots as well as rates of root productivity and microbial community composition to address what factors might influence changes in soil C content through time. Between pages 8-9, we provide this explanation stating that there might be different mechanisms which can lead to more C in soils, either C inputs from animal slurries or C inputs from larger root systems as in the control plots.

Was there really a difference in C accumulation rate between pig and cattle slurry given these were largely applied at different rates (cattle rates were higher or the same than pig rates).

This is a good point and we have now clarified it describing what potential differences there might be between pig and cattle slurry applications in determining soil C gains or losses. First, we removed our emphasis on cattle slurry applications as being more beneficial to soil C sequestration than pig slurry applications. Second, we clarify that while all cattle slurry applications were associated with significant changes in soil C accumulation rates when compared to control and NPK plots, pig slurry applications were not. Third, we explain why absolute and relative effects of cattle and pig slurries on soil C sequestration might differ (please see below and also on page 7, section 4.1 of the discussion).

Expanded below. Specific points:

1. In the site description, the authors state the site was established in 1970 on an existing sward of ryegrass clover. If it has been in pasture for decades previously but still increasing in soil C stocks this would seem very odd. Was the site cropped in the past and so still recovering from previous C loss? I understand that getting previous land use can be difficult but this is important as the increase in soil C is up to 20% of initial stock in the control soils in 40 years (gain of 13 Mg from a base of about 59 Mg).

We have now added information about a ploughing and reseeded event occurred in 1969. See page, lines 7-9: “The Long-Term Slurry experiment (LTS) was established in 1970 on a pre-existing sward of perennial ryegrass, which was previously established in 1969 after a ploughing and reseeded event”. We agree that net C change in control unfertilized soils has been significant and we don’t know for sure whether this is related to potential soil C losses following the reseeded event in 1969. On a parallel study (Carolan R & Fornara DA. 2016. Soil carbon cycling and storage along a chronosequence of re-seeded grasslands: do soil carbon stocks increase with grassland age? *Agriculture, Ecosystems and Environment* 218, 126–132) we find that reseeded actually determines a short-term increase in CO₂ fluxes from soils. If this was the case in our permanent grassland, it has been taking more than 43 years to ‘replenish’ the soil C pool. I think this potential ‘saturation process’ deserves more research using long-term experiments.

2. Figure 2b. All the replicates are plotted which presumably gives the tight error bounds, is this reasonable? Error bands are not defined in legend.

We have changed Fig. 2b following the reviewer indications. We now show mean values and associated standard error bars. We also modified the legend of Fig. 2 as following: “Fig. 2. Relationship between net soil C sequestration rates ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$; 0-15 cm depth) and (a) nutrient application treatments, (b) C inputs from animal slurry ($\text{Mg C ha}^{-1} \text{ yr}^{-1}$). Experimental treatments same as in Fig. 1. Symbols: Square = no C inputs (control plots), Circles = C inputs from pig slurry, Triangles = C inputs from cattle slurry. Standard errors indicate variation among six replicate values for each treatment”.

3. Figure 2b. I am not sure that the authors can assume a linear fit – to me a broken stick model could be fitted that is essentially flat to inputs of about 1.2 Mg C/y and then increases. If correct this could simplify interpretation of why pig and cattle slurry gave different responses. A broken stick model or similar curve would suggest that the first C load of added C is mineralised and the remainder is available for stabilisation. This is an important distinction, the current figure could be interpreted to mean that any addition of external C will build soil C but a broken stick model (or similar model) would argue that there is a threshold load that is needed before C accumulates. I would have thought the authors need to defend the linear fit. This curve is strongly dependent on the two high C loading from cattle and so the discussion about differences in composition of cattle and pig manure leading to different carbon accumulation (first three paragraphs of the discussion) might more easily be explained by a lower loading rate of pig slurry relative to cattle slurry.

We perfectly agree. The linear fit is probably not justified also because pig and cattle slurry applications may return to the soil C compounds with different biochemical properties. We thus prefer not to fit any model but show the different rates of C additions under each slurry type (pig and cattle) and their variation using standard error bars. Note that we have introduced symbols to characterize the two types of slurry in Fig. 2b. We think that the new Fig. 2b fits very well with the three potential explanations (under section 4.1 in the Discussion, pages 7 and 8), that we give to describe why cattle and pig slurry applications may influence soil C sequestration differently.

4. The highest pig slurry loading was 1.11 Mg C/ha/y in comparison to lowest cattle slurry application of 0.92 Mg/ha/y both of which had standard errors of about 0.1 (Table 1 – I think these are SE - not stated). Are these significantly different loads? The relative soil C stock changes of 1.05 for pig slurry and 1.09 for cattle slurry with SE of 0.04 and 0.03, respectively (table 1). So for the same slurry load from pigs and cattle gave same amount of C accumulation and no need to try to justify a difference between cattle and pig slurry? Looking at fig 2b the slightly higher C accumulation for the cattle slurry at 0.92 Mg C/ha/y inputs is driven by one high point – the other three points fit within the scatter of the slurry C input of 1.11 Mg C/ha/y. It is important to be clear about this otherwise the reader might conclude that there were indeed differences in C accumulation for cattle and pig slurry when I think this is hardtop justify.

This is a good point indeed and we have now better clarified what potential differences there might be between pig and cattle slurry applications in determining soil C gains or losses. First, we removed our emphasis on cattle slurry applications as being more

beneficial to soil C sequestration than pig slurry applications. Second, we clarify that while all cattle slurry applications were associated with significant changes in soil C accumulation rates when compared to control and NPK plots, pig slurry applications were not. Third, we explain why absolute and relative effects of cattle and pig slurries on soil C sequestration might differ.

Please see new modified paragraphs on pages 7-8: “Our results show that rates of C accumulation in top-soils significantly increased under high rates of cattle slurry applications. Significant accumulation of C in soils also occurred under low application rates of cattle slurry when compared to control or to NPK nutrient treatments. Instead, pig slurry applications were not associated with significant changes in soil C sequestration rates when compared to unfertilized soils or to soils receiving inorganic (NPK) nutrient additions. Soil C changes in response to pig slurry applications were not as linear as those observed under cattle slurry applications (Fig. 2a, b). We suggest that both absolute and relative effects of cattle vs. pig slurry applications on soil C sequestration will depend on different factors (e.g. animal diets, rates of slurry applications, slurry biochemical composition etc.) and can thus have different explanations.

First, the amount of C added to soils through cattle slurry in our study is higher than in pig slurry (Table 1), and we found a strong positive correlation between C inputs to soils and net soil C accumulation (Fig. 2b). Our estimate is that cattle slurry-C retention efficiency varies between 14-15%, a value that is very similar to a global manure-C retention coefficient of 12% estimated in a recent meta-analysis study (Maillard and Angers, 2014). This means that 85-86% of all C applied yearly to our soils is lost potentially via increased soil CO₂ fluxes and/or organic C leaching from soils. However, 15% C retention efficiency associated with high rates of cattle slurry significantly contributed to soil C accrual in this long-term grassland experiment.

Second, the biochemical composition of cattle and pig slurries may be significantly different. We did not find any significant difference in total N% (2.9 ± 0.9 vs. 3.1 ± 0.7) or total C% (32.3 ± 2.8 vs. 34.8 ± 3.7) content between pig and cattle slurry, respectively. However, animal ruminants such as cattle are particularly efficient in using C components in their grass feed and excrete high concentrations of slowly digestible organic matter including lignocelluloses (Van Kessel et al., 2000). It is well established that lignin concentrations are negatively related to the biodegradability of organic material in animal manures (Triolo et al., 2011), and that high lignin-to-N ratios tend to slow organic transformations in soils (Chantigny et al., 2002) and can thus lead to greater organic C accumulation in grassland soils (Parton et al., 1987). Recent evidence, however suggests that the concentration of lignin and other biochemical compounds either in animal slurries or in different organic amendments are not necessarily good predictors of their long-term residence time in soils (Lashermes et al., 2009).

Thirdly, long-term pig slurry applications were generally associated with poor soil C and N retention per unit of N added (Fig. 4a, b). Here soil C and N retention rates were higher under low rates of pig slurry applications suggesting that increasing application rates of pig slurry may contribute to higher C losses from soils. Previous studies have shown that pig slurry applications can cause soil C losses either by significantly increasing soil CO₂ fluxes (Rochette et al., 2000) or by promoting a ‘priming effect’ whereby high biodegradable pig slurry applications stimulate the mineralization of both native soil C and fresh root-derived material (Angers et al., 2010). Given the high

variability in the concentration and abundance of both recalcitrant (e.g. lignin) and labile (e.g. volatile fatty acids) organic compounds in pig and cattle slurries more studies are required to address potential linkages between slurry biochemistry and long-term C and N transformations in soils”.

We have also described the meaning of standard error in Table 1. “Effects of C application rates of different animal slurries to soils on (1) Slurry C-retention coefficient, and on (2) Relative soil C stocks change. The ‘Slurry-C retention coefficient’ represents the average proportion of slurry-C, which has been annually ‘retained’ in soils after 43 years of slurry applications. The ‘Relative soil C stocks change’ is the ratio between SOC stocks in plots receiving slurry and SOC stocks in plots receiving mineral (i.e. NPK) fertilization (*sensu* Maillard and Angers, 2014). Standard errors indicate variation among six replicate values for each treatment”.

5. Figures in general need attention – superscripting missing in some (e.g., fig 4a) and not used in some places (e.g., axis fig 2b), different fonts e.g., figure 6. Figure titles don’t state what error bars are. Figure 1 what is dashed line – an overall linear fit?

We have now edited all figures in the main manuscript as well as figures in the Supplementary material. Superscripts have now been added to title axes, fonts were homogenized across figures. We have also added information on the meaning of our standard errors in all figure legends. Finally we specify that in Fig. 1 the dashed lines represent linear fit functions.

6. Pg 3 (ln 34-36) %C was measured using LECO and then also by muffle furnace – please explain why two different measurements approaches were used– is this to get at inorganic C?

Yes, we wanted to assess how much inorganic C% was included in our samples. We have now specified that: “To determine inorganic C concentration, bulk soil subsamples were burned for 16 h at 550 °C in a muffle furnace and ashes analysed for inorganic C and N content” (page 3, lines 36-37).

7. Pg 5 ln 7-10. Was the accumulation rate from the control subtracted before calculating Slurry retention factor? I see this is stated in table 1 but missed it in data analysis section.

Yes, this part was not well clarified in our Data Analysis section. We have now added one more sentence to this section: “The difference in total soil C content between each slurry-fertilized plot and the average of the unfertilized controls was also divided by C added over 43 years under each slurry application. We thus calculated the ‘Slurry-C retention coefficient’ (*sensu* Maillard and Angers, 2014), which is the average proportion of slurry-C, which has been annually ‘retained’ in soils after 43 years of slurry applications. Data were analyzed using JMP version 9.0.0 (SAS Institute, Cary, North Carolina, USA)” (page 5, lines 9-13)

8. Pg 5 ln 7-10. I guess this assumes that the extra stored C comes only from the

slurry but there was increased plant production also and this has potential to be stabilised in soils also? How was this accounted for? Pg 6 ln 19 states that 16% of slurry C was accumulated in soils but does this ignore the extra pasture production and inputs? But pg 7 ln 36 states 14-15% retention.

Yes, here we refer to the ability of these soils to retain C under specific animal slurry applications. It might be that aboveground plant biomass production has contributed over time to additional C inputs to soils but this is very difficult to assess mainly because most of aboveground plant biomass is removed every year during the three annual cuts. Our evidence is that potential C inputs from aboveground plant biomass don't seem to have any significant effect on changes in soil C through time. For example, the productivity of control plots is much lower than NPK fertilized plots (Fig. 5a), however, soil C accumulation seems to be similar or even higher in the control-unfertilized soils (Fig. 1, Fig. 2a). Also high pig slurry applications led to similar or even higher productivity compared to high cattle slurry applications (Fig. 5) but increases in soil C are significantly higher in the cattle slurry fertilized soils. It would be very interesting to measure how much plant litter is returned to soils every year under the different treatments, our impression however is that C inputs from aboveground plant biomass in these managed grasslands could be very limited.

9. Pg 7 ln 10 rounding error? should be 10 Mg?

Yes, thanks we have now corrected this. See: "GWP estimated for beef farming ranged from 913 to 952 g CO_{2-eq} m⁻² yr⁻¹ (i.e. 9.1 to 9.5 Mg CO_{2-eq} ha⁻¹ yr⁻¹) under increasing cattle slurry applications and 997 g CO_{2-eq} m⁻² yr⁻¹ (i.e. 10 Mg CO_{2-eq} ha⁻¹ yr⁻¹) under NPK applications".

10. Pg 8 ln 31 – I do not understand sentence starting “As opposite . . .

We agree this is not clear and we simply removed “As opposite” from the sentence. See page 9, line 2.

11. Conclusions – I suggest need to tone down the statement “Our findings suggest that permanent grasslands act as a sink rather than source. . .” this may well be true for the current study at present but other have in some cases found losses of C from pasture soils:

Schipper et al. (2014), (Meersmans et al., 2009, van Wesemael et al., 2010) for specific soil types. I also think that reference should be made to saturation likely occurring at some stage even with ongoing manure inputs – I think this has been well demonstrated for some of the long-term manure experiments at Rothamsted (e.g. Johnston et al., 2009).

We agree with the reviewer and we have now edited this section (pages 9-10). We state: “Our findings suggest that permanent grassland soils may act as sinks of atmospheric CO₂ and that an equilibrium state has not been reached after 43 years. However, previous studies show that permanent grassland soils may actually lose C in the long-term (Schipper et al., 2014) and that grassland soils will eventually reach a C saturation point under long-term animal manure applications (Johnston et al., 2009). The soil C accrual

observed in our grassland may contribute to reducing the C-footprint of key commodities (i.e. milk)”...

We have also added to our reference list two recent studies: Schipper et al., 2014; Johnston et al., 2009.

12. I would strongly encourage the authors to provide numeric data rather than just bar graphs, if others want to use this data for comparison or modelling purposes bar graphs are not helpful as you have read off the graphs. Numeric data could be provided in supplementary materials.

We made sure that key rates of soil C and N change through time under different nutrient treatments as well as values of plant productivity and total root mass are reported throughout the Results section. All values are associated with standard error values as well.

References

Johnston AE, Poulton PR, Coleman K (2009) Soil organic matter: its importance in sustainable agriculture and carbon dioxide fluxes. In: Advances in Agronomy, Vol 101. (ed Sparks DL) pp 1-57.

Meersmans J, Van Wesemael B, De Ridder F, Dotti MF, De Baets S, Van Molle M (2009) Changes in organic carbon distribution with depth in agricultural soils in northern Belgium, 1960-2006. Global Change Biology, 15, 2739-2750.

Schipper LA, Parfitt RL, Fraser S, Littler RA, Baisden WT, Ross C (2014) Soil order and grazing management effects on changes in soil C and N in New Zealand pastures. Agriculture Ecosystems & Environment, 184, 67-75.

Van Wesemael B, Paustian K, Meersmans J, Goidts E, Barancikova G, Easter M (2010) Agricultural management explains historic changes in regional soil carbon stocks. Proceedings of the National Academy of Sciences of the United States of America, 107, 14926-14930.