Spatial patterns of CO₂ fluxes across litter amended, non-amended, and native soils on cotton farms in southern NSW

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Abstract

Soil carbon dioxide (CO_2) flux can be an important indicator of soil biological and geochemical processes, often reflecting modern-day carbon (C) cycling. In southern NSW, poultry litter (PL) is a widely adopted amendment for cotton farms to improve soil physical and biological health. However, few studies have quantified the effect on soil C cycling across irrigated landscapes. We investigated the spatial patterns of soil CO_2 fluxes and C in cotton fields, with and without a history of PL, and adjacent native areas, across 10 farms in the Murrumbidgee Valley. Overall, land use was not a significant driver of CO_2 flux, yet significant within-farm variability was observed between natural, PL, and non-amended fields. Mixed effects modelling showed CO_2 fluxes were best predicted by manganese (Mn), extractable C to inorganic nitrogen ratio, and soil moisture, which suggested soils, irrespective of history, were in an advanced state of organic matter decay. Other indicators of soil C and microbial health revealed that most cultivated sites were depleted in available C, yet PL sites contained significantly higher Mn. Our results suggest Mn plays a key role in soil carbon decomposition in southern cotton soils.

Key words
Soil respiration, agroecosystems, land-use, organic amendments, irrigation, Riverina

Introduction
In the Australian Riverina district, poultry litter (PL) is becoming a widely adopted soil amendment by cotton growers, often used for improving soil physical structure and for providing supplemental nutrients (Quayle, 2018). The close proximity of poultry production to farms makes PL an ideal resource for improving soil health and supports a closed-loop resource farming system. In addition, PL provides a substantial organic matter (OM) source, yet the effect this has on soil carbon (C) processes up has not been documented for intensive irrigated soils. With the Australian cotton industry developing sustainability targets around reducing their C footprint (CRDC, 2019) there exists a need to investigate management strategies for achieving on-farm carbon neutrality.

Soil carbon dioxide (CO_2) flux and microbial biomass are key indicators of current soil C mineralisation and organic matter decomposition due to their sensitivity to soil physical and chemical changes (Cheng *et al*., 2013). As such, soil CO_2 flux can vary significantly between ecosystems in response to the quality and quantity of litter input (Raich and Tufekciogul, 2000). A number of field studies have demonstrated that manure amendments can affect organic C pools and alter decomposition rates, with mixed results on whether continuous inputs can increase C accumulation over time (Khaliq and Abbasi, 2015; McMullen *et al*., 2015). However, experimental studies are generally localised, which limits the ability to quantify impacts across regional scales.

This pilot study aimed to capture the spatial variability of soil CO_2 flux and microbial health of southern cotton growing regions. Our objectives were to: i) determine the environmental drivers of soil CO_2 flux to gain insights on factors driving organic C decomposition, and ii) assess if cultivation and the practice of PL amendment has a legacy effect on soil C and microbial indicators across the region.

Materials and Methods
The study was conducted in the Murrumbidgee and Coleambally irrigation areas in southern NSW, Australia, immediately prior to the 2020-2021 cotton-growing season. Ten representative commercial cotton farms were selected from across the farming region, and three adjacent land use types, including fields with a PL history, no-PL history, and native areas, were targeted for soil CO_2 flux measurements and sampling. A total of 27 sites were identified for this study. Although most farms were primarily used for cotton, alternating cropping systems included wheat, barley, corn, rice, and chickpeas. The uncultivated native areas consisted of river red gum and grasses, remaining mostly free of human disturbance, with some open to

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livestock grazing. Most cultivated field sites were bare soil. The primary soil types were vertosols, sodosols, and chromosols (Hornbuckle *et al*., 2008), with an average annual rainfall of 407 mm.

Table 1: Mean ± standard deviation of selected soil (0-15 cm) properties of native, PL and no-PL amended sites.

Land-use	WHC (%)	EC$_{water}$ (dS m^{-1})	C/N ratio	Total N (%)	Inorganic C (%)	SOM (%)	CEC (Cmol$_+$ kg^{-1})
Native	66.8 ± 9.5	0.12 ± 0.01	13.9 ± 1.57	0.12 ± 0.01	0.12 ± 0.04	2.93 ± 0.24	18.2 ± 1.35
PL	71.8 ± 8.5	0.20 ± 0.01	11.6 ± 0.88	0.10 ± 0.01	0.08 ± 0.01	2.13 ± 0.11	23.8 ± 1.02
No PL	74.4 ± 10.0	0.17 ± 0.01	12.0 ± 1.84	0.09 ± 0.01	0.11 ± 0.02	1.77 ± 0.09	26.2 ± 1.24

WHC: water holding capacity, EC: electrical conductivity, SOM: soil organic matter, CEC: cation exchange capacity

Soil CO_2 fluxes were measured using a portable infrared gas analyser (LI-8100) equipped with an automated survey chamber (LI-8100-103, LI-COR, Lincoln, Nebraska, USA). One day prior to measurements, three chamber collars (20.3 x 15 cm*)* placed 3 m apart were pushed 5 cm into the soil at each site. At native sites, collars were placed in sparsely vegetated areas and any live plants were removed to prevent plant respiration. Soil CO_2 flux was measured between 10:00 and 15:00 at 60 sec incubations, which were repeated twice on each of the three replicates to ensure steady state conditions. Soil (0-10 cm) temperature and volumetric water content was measured concurrently with the LI-8100 probe.

Three soil cores (0-15 cm) were collected at each PL, no-PL, and native site on each farm following CO_2 flux measurement (total 81 soil cores). Each core was dried at 60°C for 3-4 days, ground, and passed through a 2-mm sieve before all soil analyses (Macdonald *et al.,* 2017). The procedure for soil microbial biomass carbon (MBC) was performed in-house using the chloroform fumigation-extraction method (Franzluebbers and Pershing, 2020), with extracts for dissolved C and N sent to Environmental Analysis Laboratory (EAL, Lismore, Australia) for analysis. All other chemical and physical analysis was performed at the EAL using methods from Rayment and Lyons (2011).

A linear mixed-effect model (LMEM) was used to explore biological, chemical, or physical drivers of CO_2 flux variability, using the R package 'lme4' (Bates *et al.* 2015, DOI: 10.18637/jss.v067.i01) with site as a random effect. Model fit was evaluated using Akaike's Information Criterion (AIC) and the conditional R^2 value for goodness of fit. A one-way ANOVA with Tukey post hoc tests was used to identify differences in soil variables between land management. All statistical analyse were conducted using R version 4.0.3.

Results and Discussion
Assessing the drivers of soil CO_2 flux reveals insights on the mechanisms of OM decomposition. Here, the LMEM showed that soil CO_2 flux during sampling was driven by manganese (Mn) and the extractable dissolved organic carbon (DOC) to total inorganic nitrogen (NO_3 + NH_4, TIN) ratio (Figure 1). Soil CO_2 flux was weakly correlated with soil moisture, while temperature revealed no significant effect, despite often being key controls of soil respiration (Raich and Tufekciogul, 2000). MBC also revealed no relationship with CO_2 flux, indicating limited microbial activity during measurement, likely associated with stressed moisture conditions. The lack of relationship with soil biological indicators may also suggest the presence of abiotic processes contributing to the net CO_2 flux (Wang *et al.,* 2020). For example, a negative flux (-0.3 µmol m^{-2} s^{-1}) was observed at a non-amended site*,* which had a high pH (8.55) and inorganic C content (0.27 %). Such alkaline conditions support the process of CO_2 uptake via carbonate weathering, usually only observed in desert soils.

The significance of the soil CO_2 flux relationship with Mn suggests that litter decomposition rates are in the latter stages of decay (Aponte *et al*., 2012), as Mn plays a key role in carbon oxidation and lignin breakdown. Mn regulates lignin breakdown via microbial Mn(II) oxidation at oxic-anoxic redox zones, a process which facilitates the breakdown of organic carbon to stabilized organic Mn-complexes producing CO_2 (Jones *et al*., 2018). The optimal pH for biotic Mn(II) oxidation to Mn(III) precipitates in soils is ~7.5 (Zhang *et al*., 2002), similar to the mean soil pH of 7.2 observed across the surveyed sites.

The positive relationship of CO_2 flux with the DOC/TIN ratio (Figure 1B) suggests a labile C source limitation. When the labile C source is depleted, microbes will turn to lignin degradation as an alternative source of substrate (Hammel, 1997). This supports the notion that soil C pools across most of the study sites are in an advanced state of decay, as indicated by the positive CO_2 to Mn relationship (Figure 1A). Soil nutrients such as N are important in regulating early rapid decay of soluble carbon substrates, yet are often

negatively related to carbon loss during the late decomposition stage (Aponte *et al*., 2012). Over time, the high concentration of N relative to available carbon can act to hinder litter decomposition and microbial activity.
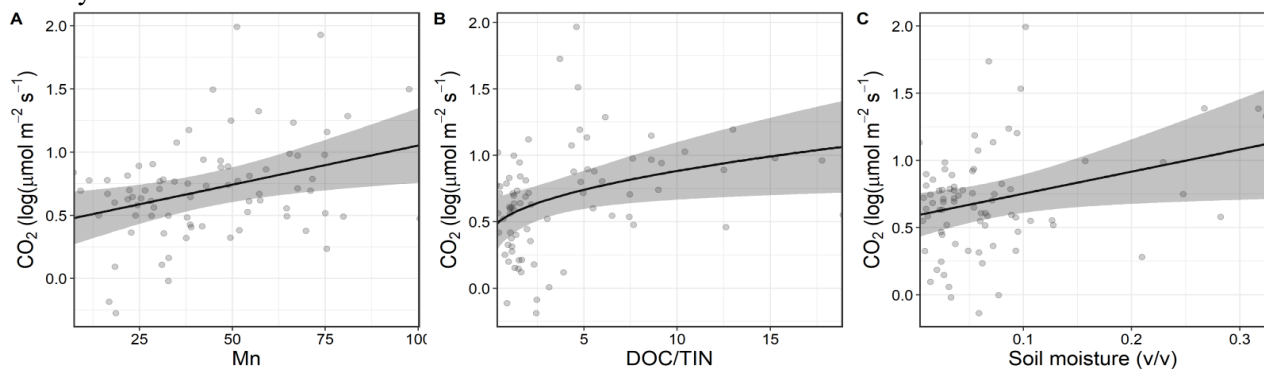

Figure 1. Partial residual plots for the LMEM showing the soil CO_2 flux response with: A) manganese (mg kg^{-1}); B) the DOC/TIN ratio; and C) volumetric soil moisture content. The conditional R^2 was 0.65.

The positive relationship of CO_2 flux with the DOC/TIN ratio (Figure 1B) suggests a labile C source limitation. When the labile C source is depleted, microbes will turn to lignin degradation as an alternative source of substrate (Hammel, 1997). This supports the notion that soil C pools across most of the study sites are in an advanced state of decay, as indicated by the positive CO_2 to Mn relationship (Figure 1A). Soil nutrients such as N are important in regulating early rapid decay of soluble carbon substrates, yet are often negatively related to carbon loss during the late decomposition stage (Aponte *et al*., 2012). Over time, the high concentration of N relative to available carbon can act to hinder litter decomposition and microbial activity.

Overall, cultivated cotton sites showed significantly higher inorganic N availability relative to labile carbon compared to native sites (Figure 2C). Organic carbon (OC) was also depleted in cultivated soils relative to native sites (Figure 2F), which is consistent with most cultivated land in Australia (Luo *et al*., 2010). This suggests that most fresh OC accumulated over the growing season had been lost, due to soil oxidation over extended fallow or residue burning, and the effect of OM addition from PL may be short lived. However, some individual farms with a long (>10 years) PL history had maintained OC levels the same as nearby native sites. Differences in microbial biomass (Figure 2D) may also reflect the low C availability observed in the cultivated soils, demonstrating a limited capacity for soil C assimilation (Hendrix *et al*., 1986).
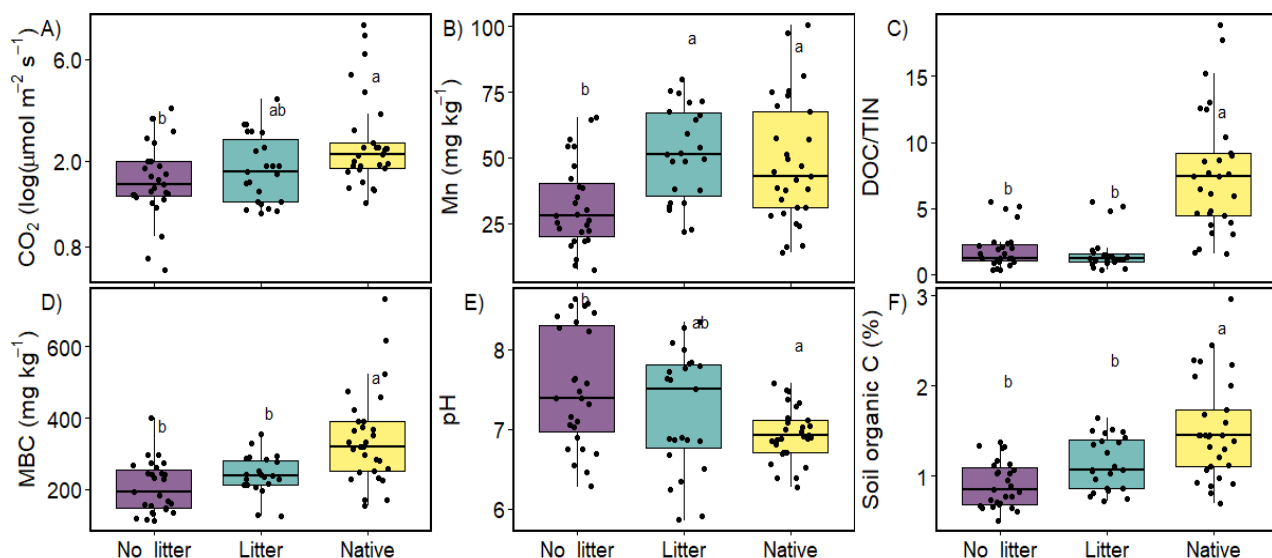

Figure 2: Box-whisker plots showing differences among soil variables between land management. Letters represent significant differences (p<0.05) among groups (one-way ANOVA and Tukey post hoc test).

Litter history only showed a significant impact on Mn concentrations, which were higher than non-litter cultivated sites and similar to levels observed in native sites (Figure 2B). Poultry manure is a rich source of micronutrients such as Mn, and has been shown to increase soil Mn levels substantially in agricultural fields (Khaliq *et al.* 2015). Given the relationship found in the LMEM, Mn provided by PL may play a key role in SOC stabilisation in irrigated agricultural soils of the Riverina region.

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Interestingly, cultivated soils were more alkaline than native sites, with a significant difference observed between non-PL amended and native sites (Figure 2E). Typically, inputs of mineral fertilisers lowers the buffering capacity of soils, yet some agricultural practices can drive changes in soil inorganic carbon stocks (Sanderman, 2012). Here, the higher pH could be due to mechanical uplifting of alkaline subsoil during land forming. Given the high soil pH conditions and negative CO_2 flux observed in one site, cultivated sites likely support the capacity for non-biological CO_2 uptake, which in some cases can offset CO_2 lost from soil respiration (Wang *et al*., 2020).

Conclusion
This observational study revealed insights into processes governing soil C loss and accumulation in Murrumbidgee Valley cotton fields. Our results showed that N availability is not a limiting factor to OC turnover in most fields, which instead were limited in labile C compared to native sites. PL amendments increased or maintained soil Mn levels at native baseline, and soil CO_2 flux results indicated that Mn plays a key role in regulating OM decomposition in these soils. This study was also one of few that found evidence for abiotic CO_2 uptake in agricultural soils: a phenomenon that requires further investigation. Future research should focus on the combined effect of different soil conservation practices with PL use to improve soil OC levels back to the native baseline.

Acknowledgements

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