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Abstract: Effects of wet/dry cycles in inducing greenhouse gas emissions are well documented. However, the effects of field drying and rewetting events remain poorly understood. This study investigated the impact of irrigation and effluent application on CO_2, N_2O and CH_4 in the Waimate District of New Zealand. Four soil management practices: (i) only added effluent, (ii) only added water, through irrigation, (iii) effluent and water added together, and (iv) neither water nor effluent added were sampled using static headspace chambers with a chamber diameter of 250 mm and height of 150 mm. All locations were sources of CO_2 and N_2O but net sinks of CH_4. Carbon dioxide fluxes ranged from 4.38 to 14.49 mg CO_2-C m^{-2} hr^{-1} while those for N_2O were between 0.007 and 0.012 mg N_2O-N m^{-2} hr^{-1}. Wetting soils receiving effluent enhanced CO_2 production by 161%, suppressed N_2O fluxes by 17% but increased CH_4 uptake by 286%. When compared with control locations, effluent-only locations observed 50% less CO_2, yet highest N_2O emissions were observed on the same locations. Nitrous oxide emissions were positively correlated with CO_2 but

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Bonface O. Manono works as a full-time lecturer in the School of Environment and Natural resource Management, South Eastern Kenya University. Prior to that, he was a full-time researcher at the Centre for Sustainability: Agriculture, Food, Energy, and Environment (University of Otago) in New Zealand where he obtained his PhD in Soil Ecology. Manono obtained his master's in Waste Management with Environmental Management from the University of the West of Scotland and a BSc in Biology from Maseno University in Kenya. Research reported in this paper reflects his future research and professional interests, thus a focus on field, theoretical and laboratory research with the aim of increasing our understanding of the soil–plant–water relationships and land management for crop production priorities and food security challenges.

PUBLIC INTEREST STATEMENT

Sustaining food production while caring for the environment is the greatest challenge facing contemporary agriculture. Intensification of farming systems negatively affects ecosystems both on and off-farm, raising concerns for major issues such as greenhouse gas emissions. This is particularly so for New Zealand dairy farming systems, which have seen continuous pressure in diversification through increased conversions and land use changes. These systems involve many components and interactions. Soil drying and wetting events and effluent application may interrupt these components and influence the gas fluxes. Not accounting for these changes may lead to under/over estimation of the gas fluxes. Earthworms and soil microbes play significant roles in redistributing nutrients, and therefore feasible interventions are necessary to optimise their use. This paper assessed the overall impact of a complete farm system on these gas fluxes under irrigation and effluent management regimes. This is important in developing agricultural mitigation strategies to reduce these emissions.

negatively correlated with CH_4 emissions. Irrigation-only locations had 33% more earthworms than effluent locations. Maximum density and biomass occurred where both effluent and irrigation were applied. There was no evidence of relationships between earthworm measurements and gas fluxes.

Subjects: Agriculture & Environmental Sciences; Agriculture; Environmental Sciences; Soil Sciences; Microbiology; Ecology - Environment Studies

Keywords: earthworm density; earthworm biomass; effluent dispersal; greenhouse gas emissions; irrigation; Waimate District; New Zealand

1. Introduction

Although agriculture is a minor emitter of CO_2, it is a major emitter of N_2O and CH_4 (US Department of Agriculture, 2004). This is a particular problem in New Zealand (Saggar, Bolan, Bhandral, Hedley, & Luo, 2004) where agriculture contributes the largest proportion of the total greenhouse gas (GHG) emissions (Ministry for the Environment, 2012). The activities of soil decomposer organisms and roots lead to production of CO_2 (Schlesinger & Andrews, 2000), while three processes lead to N_2O release from soils: oxidation of ammonia to NO_2^- and NO_3^- (Kowalchuk & Stephen, 2001); reduction of NO_3^- to NO_2^-, NO and N_2O under anoxic conditions (Knowles, 1982); and oxidation of ammonia to NO_2^- which is then reduced to N_2O, NO and NO_2 (Wrage, Velthof, van Beusichem, & Oenema, 2001). On the other hand, CH_4 emissions result from anaerobic decomposition of organic matter (Thauer, 1998), while its oxidation by methanotrophs enhances the soil's CH_4 sink capacity (Dalal, Allen, Livesley, & Richards, 2008).

The release and consumption of these gases depend on soil water content, temperature and substrate availability (Bollmann & Conrad, 1998; Philippot, Hallin, Börjesson, & Baggs, 2009) on an annual, seasonal or even daily basis (Du, Lu, & Wang, 2006). Therefore, irrigation and effluent (generated material containing cow excreta and urine diluted with wash down water after milking) application can directly or indirectly affect key variables that regulate these gas emissions from soils. Nevertheless, irrigation has received little investigation compared to contributions from other agricultural activities (King et al., 2009). Short-term emission pulses following soil drying and wetting are well described (Butterly, Bünemann, McNeill, Baldock, & Marschner, 2009; Linn & Doran, 1984; Priemé & Christensen, 2001). In general, these emissions recede in repeated drying and wetting events (Muhr, Goldberg, Borken, & Gebauer, 2008). The intensity and duration of the drying and wetting events could affect mineralisation and weathering (Borken & Matzner, 2009; Fierer & Schimel, 2002) and consequently lead to spatial and temporal emission fluctuations. Studies directly comparing GHG emissions from irrigated and un-irrigated soils report either increased emissions from irrigated soils (Horváth et al., 2010; Rochette et al., 2010), or no significant differences among them (Holst, Brüggemann, & Butterbach-Bahl, 2008).

Spreading animal waste onto land provides nutrients for pasture growth and supports rich microbial communities in soil (Elhottová et al., 2012). It also acts as a source of greenhouse gases (Liu & Greaver, 2009; Oenema et al., 2005) since they provide readily available substrates for microbial decomposition (Swift, Heal, & Anderson, 1979; Wardle et al., 2004). Soil management practices that affect earthworm communities stimulate microbial activity (McLean, Migge-Kleian, & Parkinson, 2006). Earthworm guts and associated structures (casts, burrows, middens) offer suitable microhabitats that support microbial communities (Marhan, Kandeler, & Scheu, 2007). Earthworms too have been shown to decompose more organic matter in wet soil than under dry conditions (Amador, Görres, & Savin, 2005). It is therefore important to determine the influence of these earthworms on GHG emissions from soils.

Extensive areas in New Zealand and all over the world are converting to farming principally supported by irrigation and effluent dispersal (FAO, 2010; Manono, 2014). In the Waimate District, the average wetting frequency is every 17 days (Manono & Moller, 2015). This raises two questions on

how GHG fluxes will be affected over the 17-day drying–wetting cycles in soils kept under long-term irrigation and normal stock management. Will continuous rewetting result in reduction of microbial activity and subsequent emission rates? Or will potential pulses during rewetting events outweigh this reduction and lead to increased overall fluxes?

In regard to this, this research was conducted to investigate the effect of irrigation and effluent application on CO_2, N_2O and CH_4 emissions from pasture soils and how these emissions relate to earthworm measurements. The study compared irrigated and effluent-treated locations with control locations under similar management regimes. The study aimed at determining: (i) the effects of irrigation on GHG emissions, (ii) the effects of effluent dispersal on GHG emissions, and (iii) the relationship between these emissions and earthworm measurements.

2. Materials and methods

2.1. Study area

The study was conducted in five typical dairy farms under grass pastures in The Waimate District (44°38′–44°54′ S and 170°59′–171°08′ E) of the South Canterbury region, New Zealand. The district borders the Waitaki River to the south, Pareora River to the north and the Hakataramea Valley to the West. It supports productive pastoral and cropping farming that forms a typical New Zealand agro-ecosystem landscape with irrigation on the flat areas of the Waitaki Basin and a smaller number of sheep and beef farms with irrigation on the flanking hills. Irrigation water is conveyed onto paddocks under gravity by constructing slightly sloping bays separated by low borders that direct the flow (Figure 1) while effluent is added as slurry. The study farms had an average stocking rate of 3.3 Livestock Units per Hectare, had been under irrigation and effluent management regimes for ≥10 years and were located within 10 km of each other. Soils of the study region are characterised by slow permeability, limited rooting depth and a medium to high bulk density. In the New Zealand soil classification (Hewitt, 1998) the soils are classified as mottled fragic pallic soils, while in USDA soil taxonomy (Soil Survey Staff, 1998), the soils are udic Haplustepts. The climate in Glenavy is characterised by a mean annual rainfall of 500–600 mm (Tait, Henderson, Turner, & Zheng, 2006), common droughts in the summer months and an average annual temperature of 11.2°C.

2.2. Sampling location selection

Gas samples were collected from twelve paddocks (individual farm fields)—six receiving irrigation water and six receiving both irrigation water and effluent spread across five different farms (Table 1). Each study paddocks had sufficiently large fragments of "un-irrigated land" for comparisons. This ensured that other aspects of soil management (e.g. pasture renovation, drainage, fertilisation regimes, stocking rate and grazing rotations) remained similar between sampled locations. From each study paddock, five sampling locations, three from the irrigated and two from the dry patches, were

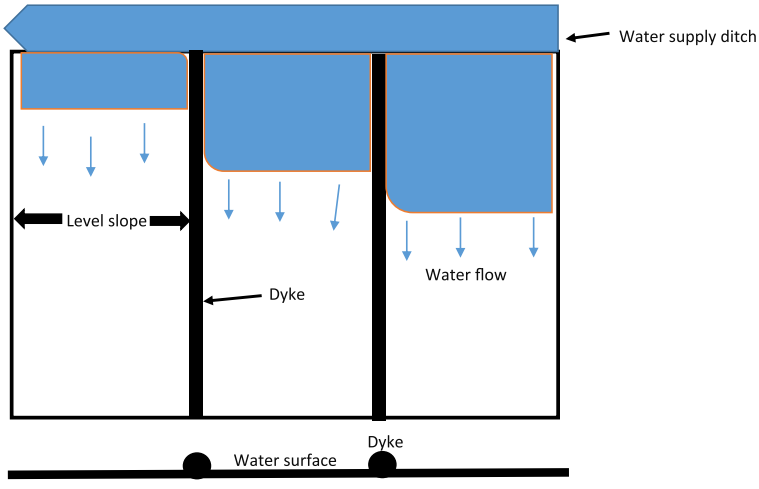

Figure 1. Schematic diagram of a typical border dyke irrigated field where a uniform sheet of water flows between dykes.

Note: Border dyke irrigation relies on passive flow of water when gates are opened between irrigation races (the water supply ditches) until a paddock is completely flooded by water.

Table 1. Sampling plan showing the total number of gas sampling locations for each soil management treatment in each irrigation cycle. Gas was collected in two consecutive irrigation cycles

Paddock management	Irrigated		Effluent and irrigated	
Number of paddocks	6		6	
Sampling location treatment	Control	Irrigation only	Effluent only	Effluent and irrigation
Number of locations	12	15	12	15

randomly selected. To exclude confounding factors, such as areas where stock congregate and trample the soil, gas collection locations were always at least 30 m away from each other, trees, fences, gateways and water troughs.

2.3. Gas sampling and analysis

Gas fluxes were sampled over the 17-day irrigation cycle in the summer of 2013. Static headspace chambers with a chamber diameter of 250 mm and height of 150 mm were used for gas collection. The methodology described by de Klein, Barton, Sherlock, Li, and Littlejohn (2003) was followed. In order to minimise the inherent issues related to chamber-based gas measurements, the chambers had vented tubes and were covered with reflective insulators (Parkin, Mosier, Smith, et al., 2003). Short headspace time interval provides a more linear curve (de Klein et al., 2003) when compared with an extended time (Parkin & Venterea, 2010; Rochette, 2011). Therefore, a 30-min deployment time was considered appropriate for this study. Actual gas sampling corresponded with irrigation events and aligned with the 17-day irrigation schedule. The first gas samples were collected on the day after soil wetting and then every fourth day until the fifteenth day. Sampling was done between 11.00–15.00 h. In total, four gas samples were collected from each location over the experimental period.

Since fluxes were expected to be linear, two gas samples were collected, the first for time 0 (t_0) taken immediately after placing the chamber and the second for time 1 (t_1) after 30 min. A Polypropylene syringe (20 ml capacity) was used to draw gas samples through a three-way stopcock directly via double-wadded caps into glass vials (6 ml Labco Exetainers®, Labco Ltd, Lampeter, Ceredigion, UK www.labco.co.uk). The gas was flushed through at least three times to ensure that the collected gas in the vial was over-pressurised. Gas samples were stored at room temperature before transporting them to the laboratory for analysis. In the laboratory, the exetainers were equilibrated to atmospheric pressure and then CO_2, CH_4 and N_2O concentrations determined using gas chromatography (SRI Instruments, California, USA) Model 8610C attached to a Gilson 222XL Auto sampler. CH_4 was determined by a flame ionisation detector (FID) analyser, CO_2 was converted to CH_4 by a methaniser and then analysed while N_2O was determined by an electron capture detector (ECD). The gas chromatography was controlled by the SRI Peaksimple software for Windows (SRI Instruments Europe GmbH). Gas fluxes were quantified in parts per million (µl/l volume).

To calculate the actual fluxes, gas concentration g(*c*) measurements were converted to mass units and corrected to field conditions by applying the Ideal Gas Law.

$$g(c) = \frac{g(v) \text{ x } M \text{ x } p}{R \text{ x } T} \quad (1)$$

where *g*(*c*) is the mass volume gas concentration, e.g. mg N_2O g/L enclosure; *g*(*v*) is the gas volume/volume concentration (trace gas concentration expressed as parts per million by volume e.g. µl N_2O l^{-1} enclosure; M is the molar mass of the measured gas (g mol^{-1}); P is the barometric pressure; R is the universal gas constant (0.0820575 L atm K^{-1} mol^{-1}) and T is the air temperature at the time of sampling in °K (°K = °C + 273.15).

It was assumed that fluxes $g(f)$ were constant and that the concentration (C) increased/decreased linearly over the 30 min (t). The slope of the converted concentrations was used to calculate the gas fluxes using the equation:

$$g(f) = \frac{V \times C}{A} \quad (2)$$

where $g(f)$ is the gas flux as mass m^{-2} hr^{-1}, e.g. mg N_2O-N m^{-2} hr^{-1}, V is the internal volume of the enclosure (chamber headspace) in m^3, A is the soil area covered by the enclosure in m^2, and C is the change in gas concentration over time in m^{-3} hr^{-1}, e.g. mg CO_2-C m^{-3} hr^{-1}.

Gas flux calculations followed the micrometeorological convention where positive fluxes are directed to the atmosphere and negative to the soil.

2.4. Earthworm measurements

Each time gas was collected, earthworms were also extracted from a 20 cm × 20 cm × 20 cm block of soil layer dug out using a spade so that the results can be expressed as density (numbers m^{-2}) and biomass (g m^{-2}). The soil extracted from the pit was placed onto a plastic sheet and searched for earthworms by sorting and crumbling the soil matrix by hand (Edwards & Lofty, 1977). Collected earthworms were identified to species level using external morphology keys and description provided in the literature. The collected earthworms were weighed using an electronic balance (accurate to 0.1 g). Please refer to Manono and Moller (2015) for further details.

2.5. Statistical analysis

All statistical analyses were performed using Genstat for Windows software (release 16). Emission differences between locations were analysed with a generalised linear mixed model fitted by restricted maximum likelihood (REML). Soil management levels and sampling days after soil wetting were assigned as fixed effects, and interactions between them incorporated when comparing the interaction effects. This meant that they were modelled separately as a function of treatment. To account for the lack of independence and hierarchical nature of the sampling, random effects were always nested as Farm/Paddock/Sampling location within the models. Orthogonal contrasts were calculated to quantify the effects of treatment separately as well as the differences on each sampling day. To test whether earthworms could best explain the variations in the observed emissions differences, earthworm data were assigned as fixed effects. For possible relationships between the different gas emission rates, CH_4, CO_2 and N_2O fluxes were investigated by calculating Pearson's correlation coefficients.

Preliminary models were constructed and residuals inspected to check for heteroscedasticity. The significance of predictor variables were assessed by Wald's tests. In the modelling, N_2O and CH_4 fluxes were not normally distributed and transformation was necessary. Nitrous oxide fluxes were transformed to $\log_{10,}$ while CH_4 fluxes were natural log transformed. Since earthworm biomass measures are continuous rather than discrete counts (not normally distributed), arcsine transformations (Sokal & Rohlf, 1981) were used to normalise variances within REML models by incorporating the usual blocking structure. Predicted transformed means and confidence intervals were back-transformed for reporting, but the *p*-values reflect the tests done on transformed data. Results are presented as means ± 2 × SE (standard error of differences).

3. Results

3.1. Flux patterns as influenced by land treatment and day of sampling

In general, CO_2, N_2O and CH_4 fluxes showed significant differences between treatments (Figure 2). The highest CO_2 emission rates were observed in irrigated-only locations with least emissions from effluent-only locations. In contrast, highest N_2O fluxes were observed in effluent-only treated locations and the least from the control locations. Methane emission rates varied considerably showing huge differences between treatments.

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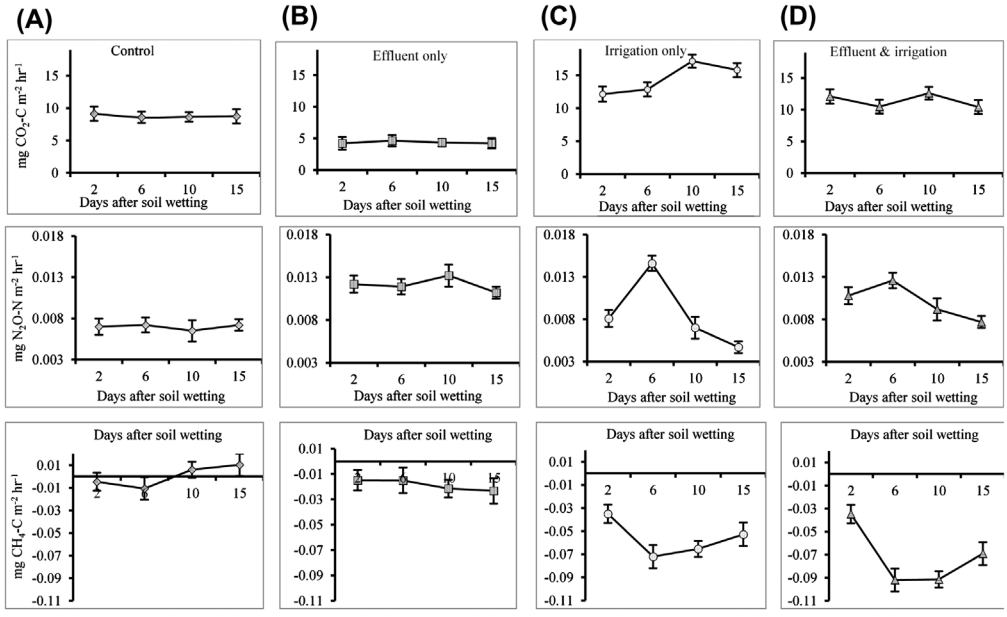

Figure 2. Mean CO_2, N_2O, and CH_4 gas emission fluxes from the interaction of soil treatment and day of sampling after soil wetting for (A) control; (B) effluent only; (C) irrigation only and (D) effluent and irrigation soil management regimes.

Notes: Values are means ± (2 × standard error of difference), $p = 0.050$ for N_2O.

Table 2. Pearson's correlation coefficients between CH_4, CO_2 and N_2O emissions

	CH_4 (mg CH_4-C m^{-2} hr^{-1})	CO_2 (mg CO_2-C m^{-2} hr^{-1})	N_2O (mg N_2O-N m^{-2} hr^{-1})
CH_4 (mg CH_4-C m^{-2} hr^{-1})	1	−0.267	−0.342
		$p = 0.012$	$p = 0.034$
CO_2 (mg CO_2-C m^{-2} hr^{-1})		1	0.165
			$p = 0.014$
N_2O (mg N_2O-N m^{-2} hr^{-1})			1

Note: The coefficients are significant at $p < 0.05$

Apart from CO_2 fluxes, temporal significant differences were observed for N_2O and CH_4 within the 15-day experimental period. N_2O emissions were highest on the sixth day after soil wetting and lowest on the fifteenth day. On the other hand, CH_4 uptake was highest on the sixth day and lowest on the second day. Nitrous oxide emissions increased immediately after soil wetting up to the sixth day before decreasing while CH_4 uptake increased immediately after soil wetting up to the sixth day before decreasing. The largest N_2O emissions were released from soils when CH_4 uptake was greatest. There was a negative correlation between CH_4 and N_2O ($R^2 = -0.342$, $p = 0.034$ (Table 2)).

3.2. Effect of the interaction between management and day of sampling on gas fluxes

The temporal heterogeneity was small and almost constant over the time of the experiment for the control and effluent-only locations (Figure 2 (A) and (B)). As the experiment was carried out during the dry summer months without rainfall, the soil moisture content was relatively uniform. Carbon dioxide and N_2O fluxes were mostly positive while soils generally acted as sinks for CH_4 throughout the measurement period. Only N_2O fluxes exhibited a significant interaction effect between soil

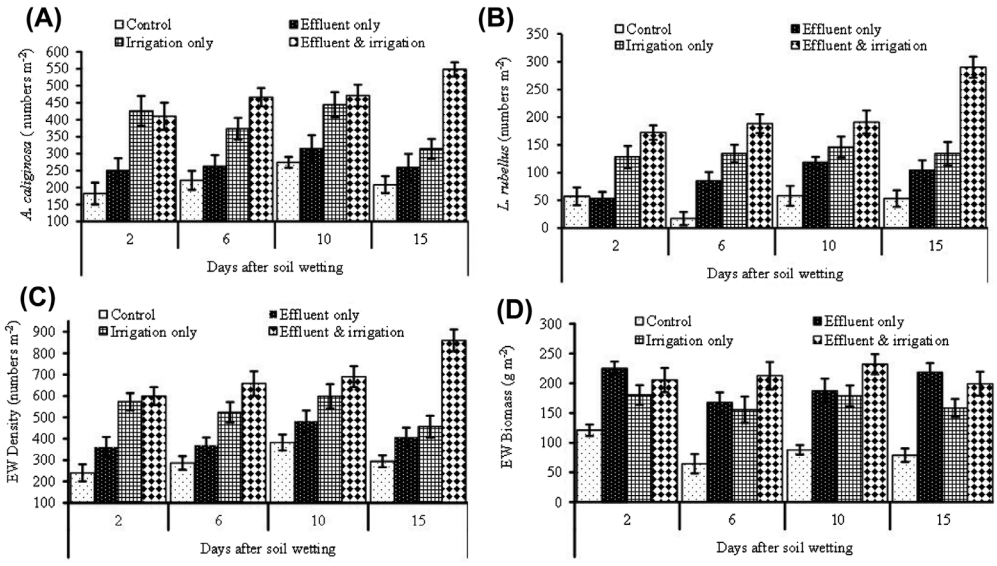

Figure 3. Mean earthworm measurements (A), *A. caliginosa*, (B), *L. rubellus*, (C), earthworm density and (D) total earthworm biomass from the interaction of soil management and day of sampling after soil wetting.

Notes: There were no significant differences for any of these measurements. Values are means ± (2 × standard error of difference).

management and day of sampling during the experimental period (Figure 2 (C) and (D)). While no obvious trends were observed for CO_2, soil wetting enhanced the soil's sink capacity for CH_4 (Figure 2 (C) and (D)). Nevertheless, these flux changes were not large enough to exhibit interaction effects for these gases.

3.3. Earthworm density and biomass as influenced by soil management

The control locations had the least earthworm density and biomasses, followed by effluent-only and then irrigation-only locations, while effluent and irrigated locations had the highest earthworm measurements. The same trend was exhibited for *Aporrectodea caliginosa* (Savigny, 1826) and *Lumbricus rubellus* (Hoffmeister, 1843) species. On the other hand, the day of sampling after soil wetting did not show significant differences for any of the earthworm measurements. The interaction between soil management and day of sampling after soil wetting did not produce any significant interaction effects for the earthworm measurements (Figure 3(A)–(D)).

3.4. Are emissions higher where there are more earthworms?

More complex Generalised Linear Mixed models were built to explore the same soil response variables for GHG emissions but with earthworms, management, days after soil wetting and the interaction between management and days after soil wetting as fixed effects. The Generalised Linear Mixed Models were of the form:

$$\text{Gas flux} \rightarrow \text{soil management} + \text{days after soil wetting} + \text{management.days after wetting} + \text{Earthworms} \quad (3)$$

This choice of model allowed the same blocking structure as incorporated in the REML models. Whereas the preceding models simply tested for independent responses for gas fluxes, these were designed to test whether gas fluxes varied for a given level of earthworms in the treatments. The full set of model parameters and constants are given in Table 3. Overall, the analysis showed no evidence that the gas flux changes were associated with a change in earthworm measures.

Table 3. Predictor parameter values in generalised linear mixed models to predict gas fluxes from the interaction of soil treatment and day of sampling after the irrigation event

Treatment		Soil property	CO_2 (mg CO_2C m^{-2} hr^{-1})	N_2O (mg N_2O-N m^{-2} hr^{-1})	CH_4 (mg CH_4-C m^{-2} hr^{-1})
Transformation used			Untransformed	Log_{10}	Natural log
		Constant	4.423	0.012	−0.014
		se	1.761	0.007	0.025
Soil treatment		Effluent only	0.000	0.000	0.000
		Untreated	**4.895*****	**−0.339***	**0.010*****
		Irrigation only	**7.902*****	**−0.161***	**−0.020*****
		Effluent and irrigation	**7.860*****	**−0.140***	**−0.020*****
		se	1.922	0.155	0.027
Sampling day after soil wetting		2nd	0.000	0.000	0.000
		6th	1.415	**−1.077****	**−0.001***
		10th	0.118	**−0.176****	**−0.007***
		15th	−0.004	**−0.134****	**−0.009***
		se	1.974	0.146	0.030
Land treatment and day of sampling interaction	Effluent only	2nd	0.000	0.000	0.000
		6th	0.000	0.000	0.000
		10th	0.000	0.000	0.000
		15th	0.000	0.000	0.000
	Untreated	2nd	0.000	0.000	0.000
		6th	−0.378	**0.124***	−0.006
		10th	−0.593	**0.022***	0.012
		15th	−0.026	**0.188***	0.024
	Irrigation only	2nd	0.000	0.000	0.000
		6th	−0.691	**0.278***	−0.057
		10th	−0.691	**0.166***	−0.032
		15th	4.665	**−0.218***	−0.019
	Effluent and irrigation	2nd	0.000	0.000	0.000
		6th	−3.040	**0.175***	−0.057
		10th	0.387	**0.108***	−0.050
		15th	−0.677	**−0.013***	−0.055
		se	2.628	0.173	0.035
Earthworms		*A. caliginosa*	−0.001	0.000	0.000
		se	0.002	0.000	0.000
		L. rubellus	0.002	0.000	−0.000
		se	0.004	0.001	0.000

Notes: se is the statistical error that is the amount by which the observed value differs from its expected value.
Parameter values are significance at *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ from REML Genstat model.

4. Discussion

4.1. Overall changes in soil gas fluxes

As reported in other studies, soils were net sources of CO_2 and N_2O emissions (Bhandral, Bolan, Saggar, & Hedley, 2007; Bolan et al., 2004; de Klein et al., 2003; Rochette et al., 2010). The exhibited N_2O pulses after soil wetting suggests that the rewetting and drying cycles did not have a

permanent effect on soil N_2O emissions. However, the regular wetting and drying may have suppressed CO_2 and CH_4 fluxes as opposed to emission pulses observed in initial soil wetting after long dry spells (Unger, Máguas, Pereira, David, & Werner, 2010; Xu, Baldocchi, & Tang, 2004). Moreover, constantly moist soil do not exhibit these flushes (Chowdhury, Marschner, & Burns, 2011). This observation is in agreement with other studies where fluxes recede in subsequent soil wetting events (Muhr et al., 2008). The enhanced fluxes in irrigated and irrigated and effluent-dispersed treatments may have resulted from increases in C and N mineralisation. This observation corroborates other emission studies in a Sonoran desert ecosystem (Sponseller, 2007); a fertilised grassland (Kim, Mishurov, & Kiely, 2010) and in a semi-arid grassland (Wu et al., 2010). On the other hand, the observation in control treatment may have been influenced by lack of water addition. Decreased soil water content lowers respiration, reduces diffusion of substrates and results in soil microbes reallocating resources potentially affecting nutrient cycling; thus, moisture limitations limit soil microbes in dry soils (Manzoni, Taylor, Richter, Porporato, & Ågren, 2012; Schimel, Balser, & Wallenstein, 2007; Stark & Firestone, 1995).

The significant flux differences observed between treatments illustrate the importance of management practices as a factor in regulating soil microbial activity in managed agro-ecosystems. However, unlike for earthworms where the effects of adding water and effluent together appear to be additive (Manono & Moller, 2015), each gas flux differed according to land treatment. For example effluent-only treated locations recorded the lowest CO_2 emissions, while the same locations recorded the highest N_2O emissions. This suggests that there is no interdependent effect of having both effluent and water added together for the three GHG emissions.

4.2. Changes in CO_2 fluxes

Carbon dioxide fluxes appeared to be associated with the level of organic additions, e.g. lower emissions from locations where effluent was applied. Unfortunately, these locations had the highest N_2O emission rates, an indication that effluent application may contribute to CO_2 mitigation but simultaneously enhance N_2O emissions. Lack of CO_2 emission differences for sampled days suggests that CO_2 fluxes are determined by long-term soil management practices rather than short-term events.

4.3. Changes in N_2O fluxes

Higher N_2O emissions from effluent-only locations corroborate other New Zealand N_2O emission studies (Barton & Schipper, 2001; Bhandral et al., 2007; Bolan, Saggar, Luo, Bhandral, & Singh, 2004; Saggar et al., 2004). One possible cause of these enhanced emissions is the exacerbation of anaerobic conditions resulting from microbial metabolism of the higher amounts of organic carbon in effluent (e.g. in one study, in New Zealand pastures, one cubic metre of effluent contained 7,400 g of total solids, 2,247 g of total carbon, 246 g of total nitrogen and 55 g of total phosphorus (Di, Cameron, Silva, Russell, & Barnett, 2002)) and the higher oxygen demand in polluted water (EPA, 1990). Since effluent is added as slurry, the increased soil water content reduces soil aeration, while increased microbial activity reduces soil oxygen availability, thus alteration of both nitrification and denitrification processes; the main processes that drive N_2O formation (Amha & Bohne, 2011; Beare, Gregorich, & St-Georges, 2009; Horváth et al., 2010). Similarly, continuous changes between wetting and drying may increase water stable aggregates, thus not easily decomposed but stored for longer time and consequently intensifying soil microbial activity (Degens & Sparling, 1995; Pulleman, Six, Uyl, Marinissen, & Jongmans, 2005). Further, mineral nitrogen contents can be higher in effluent than non-effluent locations.

4.4. Changes in CH_4 fluxes

Apart from the control location, soils consistently acted as a sink for CH_4 which is typical for temperate grassland soils (Liebig, Gross, Kronberg, & Phillips, 2010; Mosier, Delgado, Cochran, Valentine, & Parton, 1997). The lack of evidence of CH_4 flush shifts after soil wetting contributed to the

contrasting views with respect to CH_4 emission studies (Davidson, Ishida, & Nepstad, 2004). High soil water content immediately after soil wetting may have restricted oxygen diffusion into the soil which is necessary to obligate aerobic methanotrophs for oxidation to occur. This caused the dip in CH_4 uptake following soil wetting. Reduced CH_4 uptake in un-irrigated locations may have resulted from reduced metabolic activity of methanotrophs due to moisture stress. Indeed, this has been reported to occur when soil moisture is low (Kammann, Grünhage, Jäger, & Wachinger, 2001; Liu et al., 2007). Continuous moisture availability and carbon-rich effluent dispersed onto paddocks allowed for maximum CH_4 uptake.

4.5. Relationship between CO_2, N_2O and CH_4 fluxes

Soils were sources of CO_2 and N_2O and the two gases were positively correlated. This positive linkage may have resulted from shared available substrates, labile carbon and nitrogen (Bollmann & Conrad, 1998), and other common controlling factors such as water and oxygen. Similarly, shared microbial processes may have produced similar impacts on microbial processes that generate these two gases (Firestone & Davidson, 1989). The larger N_2O emissions coinciding with peak CH_4 uptake under irrigated locations that were negatively correlated indicate the occurrence of spatially separated soil microsites with contrasting environments and processes. It suggests possible shifts of N_2O along with changes in CH_4 emissions that can offer trade-off opportunities for reduced net GHG emissions and warrant further studies for this determination.

4.6. Relationship between cumulative fluxes and earthworms

Many complex and mutual interdependent relationships between earthworms and soil microbes have been observed in many studies (Drake & Horn, 2006; Lubbers, Brussaard, Otten, & Van Groenigen, 2011; Lubbers et al., 2013). However, there was no evidence of a predictive relationship between earthworm measurements and gas fluxes in the present study. This contrasted with other studies where positive correlations between earthworms and gas flux emissions have been observed. For example, those observed between CO_2 fluxes and earthworms (Caravaca, Pera, Masciandaro, Ceccanti, & Roldán, 2005) and N_2O fluxes and earthworms (Borken, Gründel, & Beese, 2000). Interactions between earthworms and other soil biotic and abiotic components may have developed several feedback disruptions that limited the impact of earthworms in this study. Earthworm presence in all treatments may have posed problems for detecting differences in gas emissions. Extreme treatments, thus; experiments with and without earthworms or microcosm experiments containing a known number of individual earthworms or known biomasses would be ideal in such a comparison.

4.7. Methodological constraints

Caution is needed when interpreting these results since the experiment was not replicated over several wetting and drying cycles or seasons. Follow-up work through several such cycles is necessary to provide greater insight into these relations. In addition, fluxes may have varied from farm to farm depending on individual farmer management. An attempt was made to minimise this disruption from the above effects by using a hierarchical and nested sampling design.

5. Conclusion

Effluent application especially on irrigated land has significant effects on GHG emissions. The environmental impact of the moderate increases on CO_2 and N_2O with irrigation is of concern, especially since increases in irrigation are expected throughout the world as part of ongoing intensification of agriculture. Lack of relationships between earthworms and GHG emissions in this study suggest the importance of examining whole systems with a longer term perspective to establish how earthworm–microbial interactions influence GHG emissions from agricultural soils.

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