N_2O and CO_2 emissions following clover and cellulose incorporation into a New Zealand pastoral soil

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Abstract
Clover (*Trifolium repens* L.) and clover + different proportions of cellulose were incorporated into soil and the nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions measured. Ground, dried clover shoots and cellulose were mixed to carbon: nitrogen (C: N) ratios of ~9 ('clover only'), 20, 30 and 40. Soil samples were incubated at water–filled pore space (WFPS) of 86% and 20^{o}C. Over 42 d, N_2O emissions from the controls averaged 9 mg/kg soil (6 g total N/kg soil), indistinguishable from the 'clover only' (1.5 g N incorporated/kg soil) and 'C: N 20' treatments. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments averaged nearly 50% greater (P <0.05) and these two treatment effects were indistinguishable. Over 42 d, CO_2 emissions from the controls averaged 4 g/kg soil. There was a linear C (incorporation rate) 'dose effect' on CO_2 emissions (0.15 g CO_2/g C, R^2 = 0.80) with no difference between clover and clover + cellulose. Over 145 d, CO_2 emissions from the controls averaged 17 g/kg soil and the C 'dose effect' was 0.38 g CO_2/g C (R^2 = 0.98). Incorporating different plant materials into soil affected the N_2O and CO_2 emissions differently.

Key Words
'Dose effect', plant litter, C: N ratio, decomposition, nitrous oxide, carbon dioxide.

Introduction
Plant litter is a complex C and N source, so its biochemical composition may affect mineralization rate. For example, plant litter of a lower C: N ratio may be more susceptible to decomposition and mineralization (Pal *et al*. 2010). Plant litter mainly contains soluble carbohydrates, cellulose, hemicellulose and lignin (in increasing order of recalcitrance) (Melillo *et al*. 1982), as well as N. Cellulose ($C_6H_{10}O_5$), an unbranched, β–(1,4)–linked, linear polymer of glucose, is a carbohydrate synthesized by plants and the most abundant organic polymer. Biodegradation of cellulose requires a distinct set of extracellular enzymes viz. cellulase, cellobiohydrolase and β–glucosidase, which act synergistically to hydrolyse the β–1,4 bonds of cellulose to glucose for further energy generation processes (Clarke 1997). Fungi including *Penicillium sp.* and *Aspergillus sp.* and bacteria such as *Streptomyces sp.* and *Pseudomonas sp.* aid in the extracellular cleavage of cellulose. The effect of cellulose in plant litter on N_2O emissions has received little attention. A potent greenhouse gas, N_2O has a global warming potential of 298 over 100 years and it is a precursor molecule involved in stratospheric ozone depletion (Forster *et al*. 2007). This paper reports the results of an experiment to measure N_2O and CO_2 emissions following clover and cellulose incorporation into soil sampled beneath pasture grazed by dairy cattle near Lincoln, New Zealand.

Methods
Litter incorporation and measurements
Temuka silt loam soil was sampled at a grazed pasture site (0–10 cm) and sieved to ≤ 4 mm. Dried, ground clover shoots and cellulose (in different proportions) were incorporated into the soil to achieve C: N ratios of ~9, 20, 30 and 40 representing 'clover only', 'C: N 20', C: N 30' and C: N 40' treatments, respectively. After treatment, soil was packed into PVC containers (internal diameter 8.0 cm, total height 10 cm) to a depth of 4.5 cm with the bottom covered by fine nylon mesh. The soil was incubated at 86% water filled pore space (WFPS) and 20^{o}C for 145 d. Emissions of N_2O and CO_2 were measured using a chamber technique with gas chromatography and infrared gas analysis, respectively.

Results and Discussion
Soil and litter properties
The θ_g, θ_v, ρ_b, and ϕ were 0.31 kg water/kg dry soil, 0.23 m^3 water/m^3 dry soil, 736 kg soil/m^3 soil and 0.72

m^3 pores/m^3 soil. The pH was 5.7, and there was 64 g/kg total C and 6 g/kg total N. The clover shoots had 51 g N/kg, 430 g C/kg, a C: N ratio of 8.5, 155 g cellulose/kg, 37 g hemicellulose/kg, and 23 g lignin/kg.

N_2O emissions

The maximum N_2O emissions were 8 h after treatment at 152.3 ± 10.1, 97.6 ± 12.9, 45.6 ± 7.6 and 21.7 ± 3.1 ng N_2O/kg soil/s (mean ± standard error of the mean, $n = 5$) for the 'C: N 40', 'C: N 30', 'C: N 20' and 'clover only' treatments, respectively (P <0.05, Figure 1). Eleven hours later, N_2O emissions from the 'C: N 40' and 'C: N 30' treatments remained significantly greater than the 'C: N 20' and 'clover only' treatments, in turn greater than the controls. Over 42 d, when N_2O emissions measurements ceased, the cumulative emissions from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control were 14.3 ± 0.5, 12.4 ± 1.2, 8.0 ± 0.6, 8.7 ± 1.2 and 9.1 ± 0.9 mg N_2O/kg soil, respectively. These emissions were in the order of control = 'clover only' = 'C: N 20' < 'C: N 30' < 'C: N 40' (P <0.05). While, unexpectedly, cumulative N_2O emissions from the controls were indistinguishable from the 'clover only' and 'C: N 20' treatments, the time courses differed significantly with 90% of the corresponding totals completed in ~38, 7 and 9 d. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments included 90% of the total completed in ~9 d. Thus, clover incorporation produced the most rapid N_2O emissions' response and adding the largest quantities of cellulose significantly enhanced the N_2O emissions response to clover incorporation.

The dry, ground clover and cellulose incorporated into the soil evidently blocked the soil pores, reducing oxygen diffusion rate and contributing to the attainment of anaerobic conditions. Higher N_2O emissions would be expected under more anaerobic conditions. The presence of available C can increase denitrification, directly, by increasing energy and electron supply to the denitrifiers, and indirectly, by enhanced microbial growth and metabolism, thereby stimulating higher O_2 consumption (Beauchamp *et al*. 1989; Gillam *et al*. 2008). The current results cannot delineate the N_2O production mechanism but given the soil moisture content and the fact that cellulose was being utilised, and that the N_2O emissions were lower than the control during 2.3–4.3 d; it is likely that the C substrate further enhanced denitrification and permitted the further reduction of N_2O to N_2 (Firestone and Tiedje 1979).

CO_2 emissions

Four hours after treatment the CO_2 emissions were 12.0 ± 0.4 < 17.5 ± 1.1 = 15.0 ± 2.2 = 11.7 ± 1.0 > 1.9 ± 0.1 µg CO_2/kg soil/s from the 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively (Figure 2). The maximum CO_2 emissions occurred at 1.4 d with 23.9 ± 0.04 > 25.1 ± 0.05 > 22.4 ± 0.17 > 18.0 ± 0.12 > 1.4 ± 0.01 µg CO_2/kg/s from 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively. The relatively low CO_2 emissions from controls suggest disturbance was not responsible for the higher emissions of treated soil; rather, the soil microbial biomass may have switched from the recalcitrant soil organic matter to the incorporated substrate (Sparling *et al.* 1982; Cheng 1996). Moreover, these higher emissions can be accounted for the so called r–strategist activity of rapid catabolism of the fresh organic matter in soil (Fontaine *et al*. 2003).

The CO_2 emissions steadily declined after 1.4 d but at 10.3–11.1 d, a further increase in CO_2 emissions was observed (Figure 2) but this increase was very minor in the 'clover only' treatment. Furthermore the 'secondary peak' in those treatments with cellulose additions was dependant on the rate of cellulose applied (i.e. 'clover only' < 'C: N 20' < 'C: N 30' = 'C: N 40') which indicated cellulose utilisation as an energy source. The 'C: N 30' and 'C: N 40' emissions did not significantly differ at 1.4 d probably because of the abundant amount of added cellulose already present in the soil. A significant 'C dose effect' was observed over the entire incubation period since the 'clover only' and 'C: N 20' treatments reached the control levels at 112.2 d followed by 'C: N 30' at 145.3 d. The cumulative CO_2 emissions over 145 d averaged 98.5 ± 3.0, 83.8 ± 2.3, 66.4 ± 0.9, 42.0 ± 1.4 and 16.6 ± 2.5 g CO_2/kg soil from 'C: N 40', 'C: N 30', 'C: N 20', 'clover only' and the control, respectively and were significantly different from one another. There was a linear 'C dose effect' (incorporation rate) on CO_2 emissions with no difference between the incorporation of clover and clover + cellulose into the soil (data not shown). Over 42 and 145 d, CO_2 emissions from the controls averaged 4 and 17 g/kg soil and the 'C dose effect' was 0.15 and 0.38 g CO_2/g C, respectively. Over 42 d, on a CO_2–equivalent basis, CO_2 emissions were ~90% of 'CO_2 + N_2O' emissions following clover and cellulose incorporation into the soil.

Cellulose occurs naturally in plant tissues and forms the basis of plant cell walls. It requires more energy to catabolise/cleave the bound cellulose for use in energy generation processes. Moreover, lignin present in the plant tissues physically protects, and therefore retards the catabolism of the bound cellulose but in the absence of lignin; it may aid the cellulose to decompose faster (Swift *et al*. 1979). We incorporated pure cellulose powder directly in the soil which although may be a recalcitrant form of C, was labile enough for the microbes as an energy source (as it was not required to be cleaved before use). Moreover, N availability can stimulate the decomposition rates (Carreiro *et al*. 2000; Geisseler and Horwath 2009). The labile–N originating from the plant litter in the present study may have stimulated the cellulose decomposition and hence caused higher CO_2 generation with an additive effect with N_2O generation.

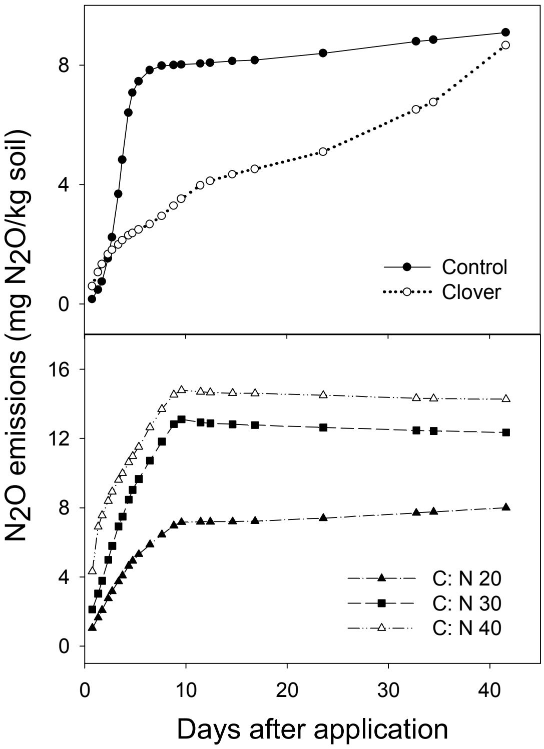

Figure 1. Cumulative N_2O emissions from 'clover only', 'C: N 20', 'C: N 30' and 'C: N 40' treatments and controls during incubation (see Methods for details).

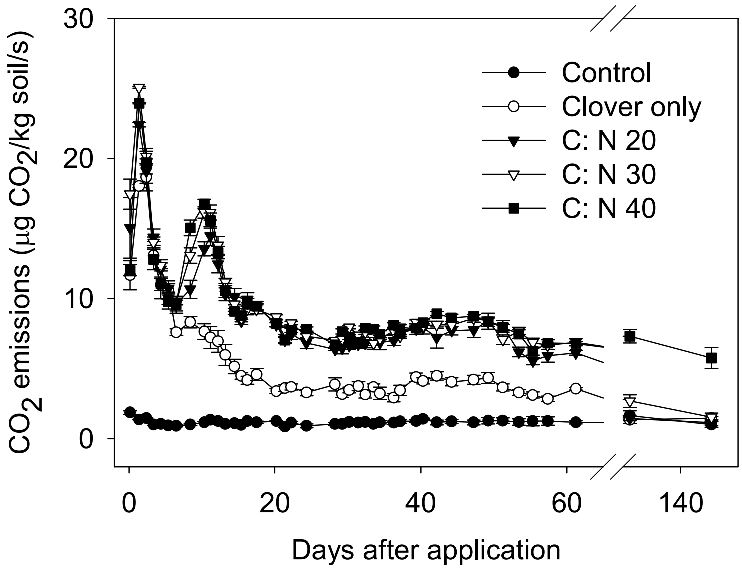

Figure 2. Soil CO_2 emissions from 'clover only', 'C: N 20', 'C: N 30' and 'C: N 40' treatments and controls during incubation. Data are means ± SE (*n* = 5) (see Methods for details).

Cellulolytic microorganisms thrive well and are enhanced in anaerobic conditions (Clarke 1997). In the present study, the high moisture content of the soil (86% WFPS) could also have produced conditions that were conducive for the cellulolytic organisms thus causing higher CO_2 emissions due to better decomposition of the incorporated cellulose.

Conclusion

Over 42 d, unexpectedly, N_2O emissions from the controls were indistinguishable from the 'clover only' and 'C: N 20' treatments. However, time courses of the N_2O emissions differed significantly; 90% of the total was completed in ~38, 7 and 9 d for controls, 'clover only' and 'C: N 20' treatments, respectively. Corresponding N_2O emissions from the 'C: N 30' and 'C: N 40' treatments averaged nearly 50% greater and 90% of the total was completed in ~9 d. Thus, clover incorporation produced the most rapid N_2O emissions response and adding the largest quantities of cellulose significantly enhanced the N_2O emissions response to clover incorporation. There was a linear C dose effect on CO_2 emissions with no difference between the incorporation of clover and clover + cellulose into the soil. Over 42 and 145 d, CO_2 emissions from the controls averaged 4 and 17 g/kg soil and the 'C dose effect' (incorporation rate) was 0.15 and 0.38 g CO_2/g C, respectively. Over 42 d, on a CO_2–equivalent basis, N_2O emissions were ~10% of 'N_2O + CO_2' emissions following clover and cellulose incorporation into the soil.

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