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Water availability and abundance of microbial groups are key determinants of greenhouse gas fluxes in a dryland forest ecosystem

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ABSTRACT

Forests are considered key biomes that could contribute to minimising global warming as they sequester carbon (C) and contribute to mitigate emissions of the potent greenhouse gases (GHG) including nitrous oxide (N_2O), methane (CH_4) and carbon dioxide (CO_2). Management practices are prevalent in forestry, particularly in dryland ecosystems, known to be water and nitrogen (N) limited. Irrigation and fertilisation are thus routinely applied to increase the yield of forest products. However, the contribution of forest management practices to current GHG budgets and consequently to soil net global warming potential (GWP) is still largely unaccounted for, particularly in dryland ecosystems. We quantified the long-term effect (six years) of irrigation and fertilisation and the impact of land-use change, from grassland to a *Eucalyptus* plantation on N_2O, CH_4 and CO_2 emissions and soil net GWP, within a dryland ecosystem. To identify biotic and abiotic drivers of GHG emissions, we explored the relationship of N_2O, CH_4 and CO_2 fluxes with soil abiotic characteristics and abundance of ammonia-oxidizers, N_2O-reducing bacteria, methanotrophs and total soil bacteria. Our results show that GHG emissions, particularly N_2O and CO_2 are constrained by water availability and both N_2O and CH_4 are constrained by N availability in the soil. We also provide evidence of functional microbial groups being key players in driving GHG emissions. Our findings illustrate that GHG emission budgets can be affected by forest management practices and provide a better mechanistic understanding for future mitigation options.

1. Introduction

Intensive anthropogenic disturbances in terrestrial ecosystems are rapidly increasing concentrations of N_2O, CH_4 and CO_2 gases released to the atmosphere, which directly affects global surface temperatures (IPCC, 2013). Soils are important sources and sinks of these three potent GHGs, with approximately 70% and 35% of the total N_2O and CH_4 emitted to the atmosphere from soils (Smith et al., 2003). In addition the complex terrestrial global C cycle is characterised by an annual emission of 120 Gigatons of CO_2, 50% of which is contributed by soil respiration (IPCC, 2013). Although both N_2O and CH_4 have lower concentrations in the atmosphere compared to CO_2, their GWP is 298 times and 34 times higher respectively, than that of CO_2 over a 100-year time horizon (IPCC, 2013). This makes them two of the most important non-CO_2 GHGs to include in future mitigation options.

Human activities can directly change GHG fluxes and alter how terrestrial ecosystems influence the climate and future GHG emission budgets. Dryland ecosystems (hyper-arid, arid, semi-arid and dry sub-humid ecosystems) are particularly important and cover about 41% of Earth's terrestrial surface (Millennium Ecosystem Assessment, 2005). They are expected to expand further by 10% globally under predicted climate change (Feng and Fu, 2013). These ecosystems are characterized by extremely low availability of soil water and nutrients, resulting from low precipitation and high evaporation (Delgado-Baquerizo et al., 2013) and hence, are considered to be highly vulnerable ecosystems. The expected expansion of dryland ecosystems not only impact human populations but can also affect current GHG fluxes from these ecosystems and further contribute to increasing GHG emissions into the atmosphere. In fact, even though studies in arid-zone soils are rare, both CH_4 oxidation (Dalal et al., 2008) as well as N_2O emissions are reported, the latter occurring mostly after summer rainfall (Barton et al., 2013). However, the mechanisms and drivers

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of GHG production and consumption in dryland ecosystems are not fully understood.

Nitrous oxide is emitted from terrestrial ecosystems through a combination of microbial processes, mostly nitrification-mediated pathways (nitrifier nitrification and/or nitrifier denitrification) and denitrification (Baggs, 2011). Nitrification-mediated pathways are facilitated by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) by aerobic oxidation of ammonia (NH_4^+) but this can also occur through the reduction of nitrite (NO_2^-) by relevant AOB (Kool et al., 2010). Denitrifying microorganisms can also generate N_2O as an intermediate or as an end product of the anaerobic respiratory pathway by reducing nitrate (NO_3^-) or NO_2^- (Baggs, 2011). More recently, work has demonstrated a N_2O sink capacity for soils via the activity of N_2O-reducing microorganisms (Jones et al., 2014). Methane production occurs through the anaerobic process methanogenesis by methanogenic archaea. It is consumed mostly through the aerobic process methanotrophy by methanotrophic bacteria, with forests known to be dominated by CH_4-oxidising microorganisms (Le Mer and Roger, 2001). Carbon dioxide is emitted through soil respiration, a combination of root, microbial and faunal respiration, and decomposition (Rastogi et al., 2002; Singh et al., 2010). All of these GHG-producing processes are primarily controlled by substrate availability, such as mineral N and labile C as well as by soil physico-chemical factors, such as pH, soil moisture, temperature and diffusivity (Dalal and Allen, 2008). These factors regulate microbial enzymatic expression which is ultimately responsible for the production and consumption of these gases (Spiro, 2012).

Forestry plantations routinely use fertilisation (N, P and K) and irrigation practices to maximise wood production by shortening rotation times. This is intended to overcome nutrient and water deficiencies that are common in many Australian, and other dryland soils. Furthermore, changes in land-use are occurring continuously, with conversion of native woodland to grazed pastures as well as conversion of pasture to forest plantations. The latter known to improve CH_4 consumption rates, reduce N_2O emissions from soil and increase C sequestration (Dalal et al., 2008; Allen et al., 2009; Livesley et al., 2009). As a consequence, some studies have addressed the impact of land-use change on GHG fluxes in Australian soils (Livesley et al., 2009; Grover et al., 2012) but much less is known about how fertilisation and irrigation affect GHG emissions from nutrient poor soils and how they alter functional microbial groups responsible for these emissions (Hu et al., 2015).

Because microbial communities play a central role in the production and mitigation of all GHGs, it is essential to understand how key functional microbial groups will respond to management practices and land-use change in order to improve the prediction of total GHG fluxes under current and future forestry management practices. In fact, knowledge of responses of GHG fluxes and their biotic drivers are practically sparse in dryland forests, with recent evidence drawing attention to the great importance of water and N availability in the net primary production and biological activity in dryland forest ecosystems (Austin et al., 2004; Delgado-Baquerizo et al., 2013). Field studies of forest fertilisation have mostly taken place in temperate and boreal forest ecosystems in the Northern Hemisphere (Levy-Booth et al., 2014) where water and nutrient limitation is less likely. Studies that link soil characteristics and N and C cycling dynamics to microbial functional dynamics are, therefore, essential for identifying the key environmental drivers of GHG fluxes in dryland forest ecosystems, and particularly to incorporate biological factors into predictive models to improve the accuracy of GHG emissions projections. This study aimed at quantifying the long-term (six years) effect of fertilisation and irrigation on N_2O, CH_4 and CO_2 emissions in a *Eucalyptus* plantation, and the impact of land-use change from grassland to forest, within a dry sub-humid ecosystem. We further identified key environmental drivers within microbial and abiotic variables from soil. In addressing these aims we hypothesized that water addition and fertilisation would favour N_2O, CH_4 and CO_2 emissions by increasing nutrient and water availability to soil microbial communities and that land-use change would help mitigate GHG emissions.

2. Materials and methods

2.1. Field site description

The experimental field study is situated at the Hawkesbury Forest Experiment (HFE) site (33°36′40″S, 150°44′26.5″E), Richmond, NSW, Australia. The field site where the experiment was established covers 5 ha and was a paddock which had been converted from native pasture grasses more than a decade earlier. The soil, a sandy loam formed on alluvial deposits is classified as Chromosol within the Clarendon formation. It is characterized by low organic matter content (0.7%) and low N (<1 mg kg^{-1}) and P (8 mg kg^{-1}) concentrations. Full soil characteristics and climate description are described in Barton et al. (2010). With a precipitation/evapotranspiration ratio of 0.6, the site is classified as a dry sub-humid environment under UNEP classification (Millennium Ecosystem Assessment, 2005).

A plantation of Sydney blue gum (*Eucalyptus saligna* Sm.) consisting of 1000 trees ha^{-1} was established in April 2007. Three different management practices, namely irrigation (I), solid fertilisation (F) and irrigation × liquid fertilisation (IF), were initiated together with a control treatment (C) which received no irrigation or fertilisation. Four experimental plots (38.5 m × 41.6 m) were replicated in a randomized block design and all trees were initially supplied with 50 g diammonium phosphate (DAP) starter blend (N 15.3%, P 8.0%, K 16.0%, S 7.7%, Ca 0.3%) to promote tree establishment. The first fertilisation event in F and IF was undertaken in January 2008 as a solid N fertiliser (N 20.6%, P 3.0%, K 7.5%, S 3.8%, Ca 4.4%) at a rate of 25 kg N ha^{-1} yr^{-1}. In October 2008, solid N fertiliser (N 21.6%, P 8.1%, K 12.0%, S 0.6%) was applied uniformly to F, and IF started with the addition of a complete liquid fertiliser (N 20.8%, P 7.9%, K 15.6%) plus liquid N fertiliser (urea-N 46%). Both treatments were at a rate of 150 kg N ha^{-1} yr^{-1} and 55 kg P ha^{-1} yr^{-1}. In I, grey water (pH 8.8, total N 0.6 mg/L, total P 3.0 mg/L) has been supplied since the establishment of the field site at a rate of 7–20 mm every 4 days, according to season and precipitation events. The irrigation rate applied to IF was the same as to I. The irrigation treatments were applied all year round, while the fertilisation treatments occur only during the growing season. In total, 16 field plots comprising 4 different experimental treatments were considered in this study, together with 4 areas of grassland (G) surrounding the forest plots in order to assess the effect of afforestation.

2.2. Greenhouse gas flux measurement

Greenhouse gas fluxes (N_2O, CH_4 and CO_2) were measured seasonally, every twelve weeks, from the beginning of May 2013 to the end of January 2014 using a static chamber technique. All sampling activities were carried out after 4 weeks of solid fertiliser application in order to avoid potential short-term effects on flux rates and microbial communities. Three polyvinyl chloride chamber anchors (diameter = 24 cm, height = 21 cm) were inserted 10 cm into the soil in each plot, between trees favouring litter areas when possible. Chamber anchors were installed 24 h before measurements were taken in order to minimise soil disturbance impact on GHG fluxes. Air samples (20 ml) were taken from the headspace (headspace volume = 4976 cm^3) after 0, 20, 40 and 60 min using a

sampling port and a plastic syringe and hypodermic needle. The gas sample was immediately injected into a pre-evacuated 10-mL glass vial (Agilent Technologies, USA) sealed with a butyl rubber stopper and aluminium seal (Sigma-Aldrich, USA). Measurements were taken between 10 am and 2 pm to minimise diurnal temperature variations. Gas samples were analysed for N_2O, CH_4 and CO_2 concentration within 1 day of sampling on a 7890A gas chromatograph with a G1888 network headspace sampler (Agilent Technologies, USA) equipped with a flame ionization detector (FID) for CH_4, a micro electron capture detector (μECD) for N_2O and a methanizer to convert CO_2 to CH_4 for detection by FID. This system uses two 1/8″ stainless steel packed columns (80/100 HayeSep Q®, Supelco, USA) and has a minimum detection limit of 0.02 ppm, 0.20 ppm and 61.41 ppm for N_2O, CH_4 and CO_2, respectively.

Fluxes were calculated for all seasons as the slope of the linear regression from the measured headspace gas concentrations with time (Matthias et al., 1980) and expressed as micrograms of N_2O–N/CH_4–C per square meter per hour (μg N_2O–N/CH_4–C m^2 h^{-1}) or milligrams of CO_2–C per square meter per hour (mg CO_2–C m^2 h^{-1}). To avoid bias against low fluxes, fluxes below minimum detectable flux (MDF) were not discarded (De Klein and Harvey, 2012). Nitrous oxide, CH_4 and CO_2 fluxes were also upscaled to yearly estimates and reported as kilograms of CO_2 equivalents per hectare per year (kg CO_2eq ha^{-1} yr^{-1}), based on a 100-year time horizon (Myhre et al., 2013), which allowed for comparison between GHG flux responses. Greenhouse gas fluxes presented as negative values represent net sink taking place in the soil. Because the treatments considered here play an essential role in determining the GHG balance of soils, further assessment of their impact on the net GWP balance was considered. This was done by calculating the contribution of the treatment-induced N_2O, CH_4 and CO_2 individual emissions to the net GWP, according to Lubbers et al. (2013). Treatments that increased GHG emission balance into the atmosphere and hence have a higher GWP are represented as positive values whereas treatments that decreased the GHG emission balance into the atmosphere are represented as negative values.

2.3. Soil sampling and physicochemical analyses

Soil temperature was measured in triplicate at 0–12 cm soil depth during GHG collection next to chambers with a portable probe (Jaycar electronics, Sydney, Australia). Soil samples were collected after GHG collection, for each of the replicate treatments during four seasons (autumn, winter, spring and summer). Two soil cores (2.0 cm diameter, 0–20 cm depth) were collected and sieved through a 2 mm-mesh sieve. Subsamples were taken and stored at −80 °C before DNA extraction. The remainder was stored at 4 °C prior to physical (particle size and gravimetric water content) and chemical (pH, NH_4^+, NO_3^-, PO_4^{3-}, total C and total N) analysis. Soil gravimetric water content was measured by drying fresh soil at 105 °C for 24 h. Soil pH was measured in a 1:5 fresh soil:mili-Q water suspension after shaking for 1 h, using a pH meter (SevenEasy pH, Metler Toledo, Switzerland). Extractable NH_4^+, NO_3^- and PO_4^{3-} concentrations in the soil were determined on a SEAL AQ2 Discrete Analyser (SEAL Analytical Inc., USA) after extraction with KCl (2 M) and Bray-1 reagent (Bray and Kurtz, 1945), respectively. Total C and N were measured on air-dried soil at the beginning and end of the experiment using a LECO macro-CN analyser (LECO, USA). Water-filled pore space (WFPS) in the top 0–20 cm of the soil was calculated based on the gravimetric soil moisture measured. In 2012, bulk density was measured after collecting soil with a stainless steel ring (length = 5 cm, diameter = 7.3 cm) with a total volume of 209.3 cm^3 and soil porosity was calculated based on bulk density and assuming a particle density of 2.65 g cm^{-3}. Soil particle size was determined following a hydrometer method (Soil Hydrometer −5 to 60 × 0.001 g/ml, Carlton Glass, Australia) (Supplementary Table S1).

2.4. DNA extraction and quantitative PCR analysis

Total genomic DNA was extracted from soil using the MoBio PowerSoil DNA Isolation Kit (Mobio Laboratories, Carlsbad, USA) according to the manufacturer's instructions, with modification of the soil weight used (0.50 g) and the initial cell-lysis step, using a FastPrep bead beating system (Bio-101, CA, USA) at a speed of 5.5 m s^{-1} for 30 s. DNA extraction yields were in the range of 10.6–61.0 ng/μL with an average of 28.8 ± 9.1 ng/μL (mean ± standard deviation) and an $A_{260/280}$ ratio of 1.95 ± 0.05 ng/μL.

Quantification of the functional genes *amoA* for nitrifying AOA and AOB, *nosZ* for N_2O-reducing bacteria and *pmoA* for methanotrophs were determined, using the following primers, respectively: crenamoA23f/crenamoA616r (Tourna et al., 2008), amoA1f/amoA2r (Rotthauwe et al., 1997), nosZ2f/nosZr (Henry et al., 2006) and pmo189f/pmo650r (Bourne et al., 2001). All primers were purchased from Integrated DNA Technologies, Australia. Quantification of the phylogenetic 16S *rRNA* gene was determined to assess total bacteria present in the soil using the primers Eub338f/Eub518r (Fierer et al., 2005). All reactions were carried out using SensiFAST SYBR No-ROX (Bioline, Australia). Each sample was quantified in duplicate in a 10 μl reaction using the BioRad C1000 Touch thermal cycler CFX96 Real-Time System (Bio-Rad Laboratories, USA). Briefly, all reaction mixtures contained 5 μl of SensiFAST SYBR No-ROX (1X), 0.2 μl of each primer (0.4 μM), 0.2 μl of BSA (0.4 mg/ml) and 2 μl of diluted template DNA (0.5 ng μL^{-1}) for all gene targets and 4 μl (1 ng μL^{-1}) for *pmoA* gene. Full details on gene-specific qPCR primer sequences and thermal cycling programs are listed in Supplementary Table S2. Calibration curves for *amoA* (AOA and AOB), *nosZ* and 16S *rRNA* gene markers using ten-fold serial dilutions were produced from cloned PCR products with plasmid pGEM-T Easy vector (Promega, Madison, USA) according to manufacturer's instructions and transformed into *Escherichia coli* strain JM109. Calibration curves for *pmoA* gene were generated from genomic DNA (*Methylosinus trichosporium*) using ten-fold serial dilutions. The presence of PCR inhibitors in DNA extracts was examined by testing different dilutions of soil DNA extract. Agarose gel electrophoresis was performed when testing standards and target DNA concentration to verify the amplification of individual PCR products of correct amplicon size. Melt curve analyses were conducted following each assay to verify the specificity of the amplification products. qPCR evaluation of the different target gene assays (qPCR efficiency range; standard R^2 values) were the following: *amoA* (AOA) (93%; ≥0.99), *amoA* (AOB) (88.2–102%; ≥0.99), *nosZ* (90–112%, ≥0.99), *pmoA* (84–95%, ≥0.99), 16S *rRNA* (95–111%, ≥0.99). During qPCR setup, evaluation and data analysis, MIQE guidelines were followed (Bustin et al., 2009). Gene copy numbers per g dry soil normalised to extraction yield were calculated for all genes.

2.5. Statistical analysis

The effect of management practices (C, F, I, IF) and land-use change (conversion of G to C) on all variables measured were tested separately, together with the effect of season, and their interactions, by repeated measures using a linear mixed effect model approach. Replicates within each experimental plot were nested within season as random effects (to account for variability at small spatial scales). When necessary, data were transformed (logarithm, square root) to fit the assumptions of normality. A Bonferroni *post-hoc* test was used for multiple pairwise comparisons. A linear regression model was then applied to each management treatment together with C (C + F; C + I; C + IF), for each GHG separately, to

explore the significance of the corresponding microbial gene abundances describing each GHG emission. Spearman rank correlation analysis was performed on each management and land-use treatment individually, together with C (C + F; C + I; C + IF; G + C) to assess possible relationships between response variables and drivers. To further explore the relative influence and effects of biotic (microbial abundance) and abiotic drivers of N_2O, CH_4 and CO_2 emissions in management practices, abiotic variables were selected for further analysis. Ammonium and NO_3^- were then combined and used as a measure of extractable inorganic N in the soil. Variables were excluded if strong intercorrelation was present (Spearman $\rho_{WFPS\ -\ gravimetric\ moisture} = 0.95$, $P < 0.001$; Spearman $\rho_{PO_4^{3-}-Inorganic\ N} = 0.60$, $P < 0.001$) as well as variables with missing seasonal data, as in the case of total C, total N and C:N ratio. Soil temperature was initially included in the analysis but due to low contribution to the variance explained and low importance as a predictor of both N_2O and CH_4 fluxes it was excluded for both gases. Transformed variables were used when appropriate to fit the assumptions of normality. A multi-model inference approach based on information theory (Burnham and Anderson, 2002) was then applied to assess the relative importance of biotic and abiotic variables in predicting N_2O, CH_4 and CO_2 emissions and the probability that a given model best describes the observed data. However, this approach was not used to identify important variables predicting GHG emissions under land-use change because there were no significant differences in N_2O and CH_4 emissions following afforestation. In the case of CO_2, because root respiration from grassland was not taken into account in the total soil respiration measured, the multi-model approach was not applied. All statistical analyses were performed with GENSTAT v16 (VSN International Limited, Hemel Hempstead, UK). Multi-model analyses were carried out using SAM 4.0 (Rangel et al., 2010).

3. Results

3.1. Effect of management practices and land-use change on N_2O, CH_4 and CO_2 fluxes

Nitrous oxide emissions were low for all treatments with no added fertiliser, namely G, C and I, and did not differ significantly between each other (Fig. 1a). Fertilisation (F and IF) significantly increased N_2O emissions compared to C treatment ($P < 0.001$; Fig. 1a). Annual N_2O emissions were 3.5 fold greater in F and 21.1 fold greater in IF compared to C treatment (24 ± 8 kg N_2O–CO_2eq ha^{-1} yr^{-1}). No seasonal effect or significant interactive effect of management practices with season was observed (Supplementary Table S3). Furthermore, in the case of G, C and I, only 23%, 33% and 38% respectively, of the fluxes measured were above MDF suggesting minimum or no N_2O emissions. Conversely, in the case of F and IF, 69% and 96% respectively, were above MDF. Nitrous oxide fluxes showed no significant effect of land-use change, season or interactive effect of land-use and season (Fig. 1a; Supplementary Table S3).

Soils were a net sink for CH_4 across all seasons, as indicated by negative fluxes (Supplementary Table S3), with G and I having all fluxes above MDF and C, F and IF with 96%, 94% and 98% respectively. Management practices had a significant impact on the strength of the CH_4 sink ($P < 0.001$; Fig. 1b); specifically the annual CH_4 sink in F was 44% lower in comparison to C, with the remaining treatments not differing significantly from each other. Season effects ($P < 0.001$; Supplementary Table S3) were also observed in CH_4 fluxes with spring showing the highest CH_4 uptake, with 44% greater CH_4 uptake in comparison to the lowest observed in winter. However no significant interactive effect of management practices and season was detected. Land-use change had no significant effect on soil CH_4 uptake but a significant season effect was observed ($P < 0.001$; Supplementary Table S3), with CH_4 uptake being 41% higher in summer compared to winter. Moreover, a significant interactive effect of land-use and season was detected ($P = 0.009$; Supplementary Table S3), with G in winter showing the lowest CH_4

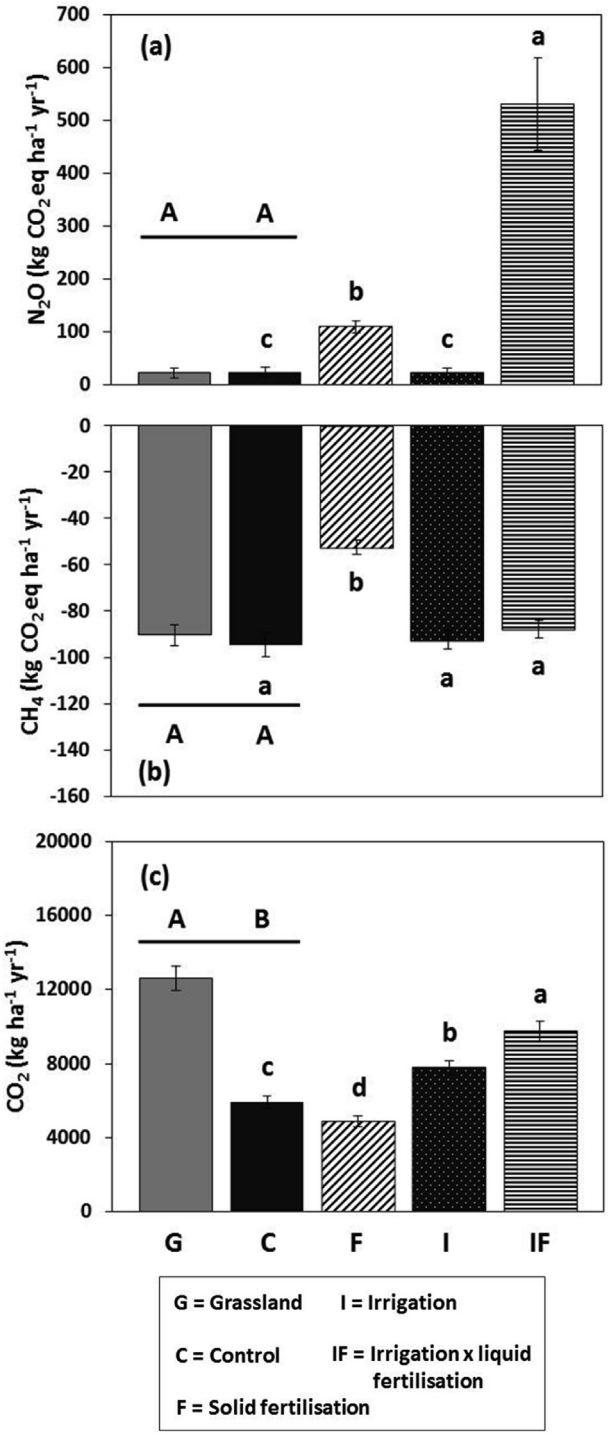


Fig. 1. Effect of management practices (C, F, I, IF) and land-use change (conversion of G to C) on (a) N_2O emissions, (b) CH_4 emissions and (c) CO_2 emissions, expressed as CO_2 equivalents per hectare per year. Values represent mean ± SEM (n = 48) of all seasons (autumn, winter, spring and summer). Statistically significant differences between management treatments (*i.e.* no grassland treatment included) are represented by different lower-case letters (a, b, c, d) and between land-use change treatments are represented by upper-case letters (A, B), according to multiple pairwise comparisons ($P < 0.05$).

uptake rates whereas the highest CH_4 uptake was observed in G in summer.

Management treatments were significantly different between each other in the case of CO_2 ($P < 0.001$; Fig. 1c), whereby CO_2 emissions were 65% greater in IF, 31% greater in I and 18% lower in F, in comparison to C. A significant seasonal effect on CO_2 emissions was evident ($P < 0.001$; Supplementary Table S3), particularly in winter where emissions were 45% lower compared to autumn and 50% lower compared to spring and summer. Significant interactive effect of management practices and season was also observed ($P = 0.03$; Supplementary Table S3), with IF during spring and summer having greater emissions in contrast to lowest emissions of F in winter. The effect of land-use change on CO_2 emissions was significantly different with C emissions 53% lower in comparison to G ($P < 0.001$; Fig. 1c), and winter emissions 41%, 42% and 49% lower compared to spring, summer and autumn, respectively($P < 0.001$; Supplementary Table S3).

3.2. Effect of management practices and land-use change on net GWP

When considering the effect of the different treatments on the net GWP balance from soil N_2O, CH_4, CO_2 fluxes, this study suggests that management practices increased the net GWP by 56% in the case of IF and 28% in the case of I in comparison to C (Fig. 2). Solid fertilisation decreased net GWP by 17%. While land-use change (afforestation) decreased the net GWP by 76% in comparison to G (Fig. 2). When assessing the contribution of each individual GHG to the net GWP (Fig. 2), overall, CO_2 had the highest contribution to the net GWP of F, I, IF and afforestation. However in F, despite the reduction of CO_2 emissions observed in this treatment leading to its net reduction of GWP, N_2O emissions contributed +9% and a reduction of CH_4 uptake contributed +5% to the net GWP. Furthermore, in IF despite CO_2 emissions being responsible for +88.2% to the net GWP, N_2O emissions contributed to +11.7%, in comparison to the negligible contribution of +0.2% from the reduction of CH_4 uptake.

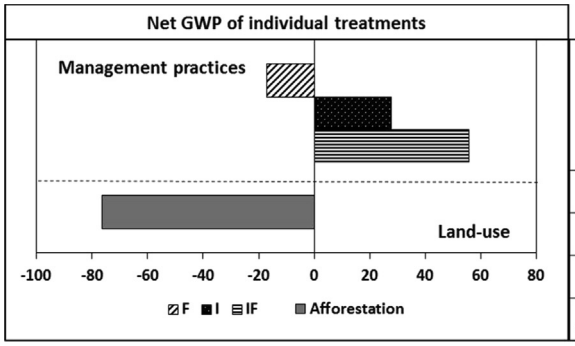
3.3. Effects of management practices and land-use change on soil abiotic characteristics

Management practices in the *Eucalyptus* plantation greatly affected soil properties (Table 1). Irrigation treatment had the highest pH with 7.8 ± 0.05, followed by IF with 7.0 ± 0.07, C with 5.5 ± 0.05 and F with the lowest pH of 4.9 ± 0.02 ($P < 0.001$). Both I and IF had over three times higher moisture contents in comparison to both C and F which had 2% moisture ($P < 0.001$). Extractable PO_4^{3-} ($P < 0.001$) was seven times higher in F, followed by IF with five times higher, and I over three times higher in comparison to C (5.9 ± 0.40 mg kg^{-1} dry soil). Ammonium concentrations ($P < 0.001$) were three times higher in F and two times lower in IF in comparison to C (1.3 ± 0.15 mg kg^{-1} dry soil), whereas NO_3^- ($P < 0.001$) was higher in both F (4.5 ± 0.27 mg kg^{-1} dry soil) and IF (3.6 ± 0.24 mg kg^{-1} dry soil), in comparison to C (0.5 ± 0.11 mg kg^{-1} dry soil). Although soil C:N ratios differed between treatments, total C and N did not ($P = 0.030$; Table 1). Ratios were highest in F (13.8 ± 0.43%) and lowest in IF (12.2 ± 0.15%). Seasonal significant differences were seen for most soil properties measured (Supplementary Table S4).

From all the soil properties measured throughout the different seasons, only gravimetric moisture ($P < 0.001$), WFPS ($P < 0.001$), NH_4^+ ($P = 0.019$), and pH ($P < 0.001$) significantly decreased after six years of land-use change from G to C (Table 1). Soil pH decreased from 6.2 ± 0.03 in G to 5.5 ± 0.05 in C, soil gravimetric moisture decreased by 50%, followed by a decrease of WFPS from 10.6% ± 1.14 to 5.3% ± 0.35 and NH_4^+ from 1.9 ± 0.17 to 1.3 ± 0.15 mg kg^{-1} dry soil (Table 1). As for management practices, significant differences between seasons were seen for most soil properties measured, except NH_4^+ and NO_3^-, pH and total C (Supplementary Table S4).

3.4. Effects of management practices and land-use change on soil microbial communities

The copy numbers of AOA *amoA* gene were consistently higher in all treatments compared to AOB *amoA* gene copy numbers (Fig. 3a and b). Management practices and season effects were significantly different for both AOA and AOB ($P < 0.001$; Supplementary Table S5). The IF treatment soils had 138% more AOA gene copy numbers and 160% greater AOB copy numbers compared to C, followed by only 32% greater copy numbers in I for AOB (Fig. 3a and b). Irrigation treatment was not significantly different in the case of AOA and F did not significantly affect AOA and AOB *amoA* gene abundances in comparison to C (Fig. 3a and b). Both AOA and AOB showed no significant differences for land-use change and only AOB had a significant effect of season on gene abundance ($P < 0.001$; Supplementary Table S5). Interactive effects



Treatment	Contribution (%) of individual GHGs to net GWP		
	N_2O	CH_4	CO_2
F	+ 9	+ 5	- 114
I	- 0.1	+ 0.1	+ 100
IF	+ 11.7	+ 0.2	+ 88.2
Afforestation	+ 0.02	- 0.06	- 99.96

F = Solid fertilisation I = Irrigation IF = Irrigation x liquid fertilisation

Fig. 2. Net GWP (%) of management practices (F, I, IF) and land-use change (afforestation) including all N_2O, CH_4 and CO_2 flux measurements in comparison to control treatments (considered here as control (C) plots for management practices and grassland (G) plots for land-use change). The table below shows the magnitude (%) of management practices and land-use change on each individual GHG flux emissions (N_2O, CH_4 and CO_2) and contribution (%) of each treatment-induced GHG emission to the net GWP, for each individual gas.

Table 1
Effect of management practices (C, F, I, IF) and land-use change (conversion of G to C) on extractable NH_4^+, NO_3^-, PO_4^{3-}, soil pH (H_2O), soil temperature, soil gravimetric moisture, WFPS, total C, total N and C:N. Values represent mean ± SEM (n = 48) of all seasons (autumn, winter, spring and summer) from 0 to 20 cm. Statistically significant differences between management treatments (i.e. no grassland treatment included) are represented by different lower-case letters (a, b, c, d) and between land-use change treatments are represented by upper-case letters (A, B), according to multiple pairwise comparisons ($P < 0.05$).

Soil abiotic properties	Treatment				
	G	C	F	I	IF
NH_4^+ (mg kg^{-1} dry soil)	1.9 ± 0.17 (A)	1.3 ± 0.15 (B) b	4.3 ± 0.45 a	1.0 ± 0.15 bc	0.7 ± 0.13 c
NO_3^- (mg kg^{-1} dry soil)	0.6 ± 0.09 (A)	0.5 ± 0.11 (A) d	4.5 ± 0.27 a	0.9 ± 0.09 c	3.6 ± 0.24 b
PO_4^{3-} (mg kg^{-1} dry soil)	4.5 ± 0.42 (A)	5.9 ± 0.40 (A) d	40.1 ± 2.22 a	18.6 ± 2.57 c	26.6 ± 1.60 b
pH (H_2O)	6.2 ± 0.03 (A)	5.5 ± 0.05 (B) c	4.9 ± 0.02 d	7.8 ± 0.05 a	7.0 ± 0.07 b
Total C (g kg^{-1} dry soil)	7.32 ± 0.19 (A)	7.65 ± 0.45 (A) a	8.67 ± 0.59 a	7.38 ± 0.16 a	7.63 ± 0.20 a
Total N (g kg^{-1} dry soil)	0.61 ± 0.01 (A)	0.60 ± 0.03 (A) a	0.62 ± 0.02 a	0.58 ± 0.01 a	0.63 ± 0.01 a
C:N (%)	12.0 ± 0.23 (A)	12.7 ± 0.33 (A) ab	13.8 ± 0.43 a	12.8 ± 0.27 ab	12.2 ± 0.15 b
Gravimetric moisture (%)	4.2 ± 0.41 (A)	2.2 ± 0.12 (B) b	2.0 ± 0.08 b	7.8 ± 0.30 a	8.0 ± 0.31 a
WFPS (%)	10.6 ± 1.14 (A)	5.3 ± 0.35 (B) b	5.2 ± 0.21 b	17.7 ± 0.70 a	18.5 ± 0.92 a
Soil Temperature (°C)	18.7 ± 0.78 (A)	17.6 ± 0.50 (B) a	17.1 ± 0.46 b	17.0 ± 0.47 bc	16.8 ± 0.48 c

of management practices/land-use with season were not significant for both AOA and AOB *amoA* gene abundances (Table Supplementary S5). Management practices significantly affected N_2O-reducing bacteria's *nosZ* gene abundance, with 65% lower copy numbers detected under F in comparison to C ($P < 0.001$; Fig. 3c). As for ammonia-oxidizers, with land-use change the *nosZ* gene abundances were not significantly different between treatments. Nonetheless, copy numbers were significantly different between season for both management practices and land-use ($P < 0.001$; Supplementary Table S5).

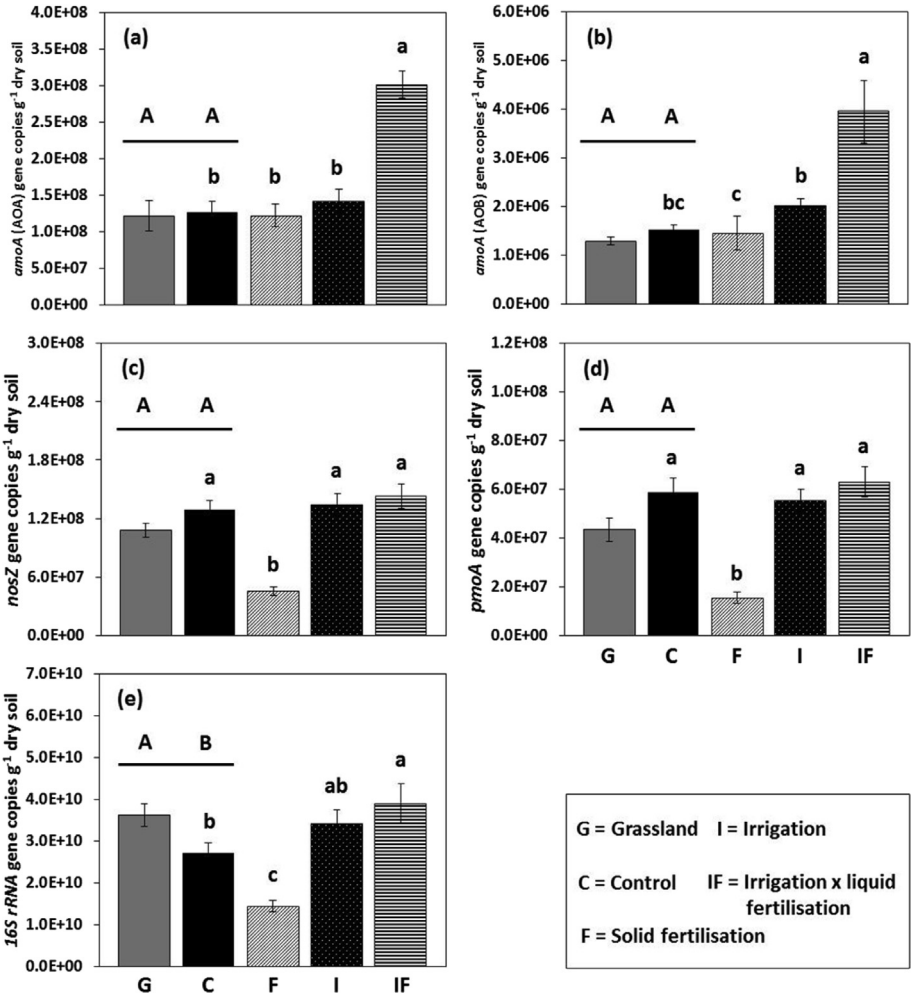


Fig. 3. Effect of management practices (C, F, I, IF) and land-use change (conversion of G to C) on various key genes involved in GHG emissions: (a) archaeal *amoA*, (b) bacterial *amoA*, (c) *nosZ*, (d) *pmoA* and (e) 16S *rRNA*, expressed as copies per gram dry soil. Values represent mean ± SEM (n = 48) of all seasons (autumn, winter, spring and summer). Statistically significant differences between management treatments (i.e. no grassland treatment included) are represented by different lower-case letters (a, b, c, d) and between land-use change treatments are represented by upper-case letters (A, B), according to multiple pairwise comparisons ($P < 0.05$).

The abundance of methanotrophs was determined by quantifying *pmoA* gene copy numbers. For management practices, F was the only treatment significantly different from C, with 74% lower *pmoA* gene abundance ($P < 0.001$; Fig. 3d). *pmoA* gene abundances were not significantly different between different land-use change treatments (Supplementary Table S5) but a 35% increase was observed in C compared to G (Fig. 3d). Seasonal differences were significant for both management practices/land-use change ($P < 0.001$; Supplementary Table S5) but no significant differences were detected for interactive effects of management practices/land-use change and season (Supplementary Table S5).

Bacterial abundance was targeted by quantifying 16S *rRNA* gene copy numbers. The IF treatment significantly increased 16S *rRNA* gene copy numbers by 43% and I increased by 25% in comparison to C ($P < 0.001$; Fig. 3e). In contrast, F had a 47% decrease in 16S *rRNA* gene abundance. Gene copy numbers significantly decreased from G to C ($P = 0.003$; Fig. 3e). Seasonal differences were observed in both management practices and land-use change treatments ($P < 0.001$) but no significant differences were detected for interactive effects of management practices/land-use change and season (Supplementary Table S5).

3.5. Relationship between microbial and abiotic factors and N_2O, CH_4 and CO_2 emissions from soil under management practices

According to the multi-model inference approach, 63 possible models were obtained for N_2O, 15 for CH_4 and 31 for CO_2, with all possible combinations of independent predictor variables. From the best fitting models that minimized AIC_c (Akaike Information Criterion corrected), the best and most parsimonious ones (smallest AIC_c and fewest variables with comparable AIC_c, respectively) describing N_2O emissions contained four and three predictor variables, respectively. The best model explained 25% of the variance found in N_2O emissions and included WFPS, inorganic N, pH and AOB abundance whereas the most parsimonious explained 23% of the variance and included WFPS, inorganic N and AOA abundance (Table 2a). When evaluating the relationship between N_2O-related genes and N_2O fluxes individually for each treatment, N_2O fluxes were significantly related to *amoA* AOA and AOB in IF ($P < 0.001$; Supplementary Figure S1c, S1f; Supplementary Table S6), whereas *nosZ* gene was not significant (Supplementary Figure S1i). On the other hand, and particularly in the case of F, only *nosZ* gene was significantly related to N_2O fluxes ($P = 0.026$; Supplementary Figure S1g). Irrigation, as expected, was not explained by either of the microbial abundances considered, probably due to low emissions detected (Supplementary Figure S1b, S1e, S1h). Ammonia-oxidizing archaea, AOB and *nosZ* gene abundances by themselves only explained 12%, 11% and 1% of the variance, respectively (Table 2a). Among all tested predictors, both WFPS and inorganic N were the most important factors explaining N_2O emissions, followed by pH whereas *nosZ* gene abundance was the predictor with least importance (Fig. 4).

The best and most parsimonious models describing CH_4 fluxes contained two and one variable, inorganic N and *pmoA* explaining 36% and *pmoA* explaining 32% of the variance found in CH_4 fluxes (Table 2b). In fact, a negative relationship of *pmoA* abundance with CH_4 fluxes was still significantly present in all treatments ($P < 0.001$; Supplementary Figure S2a, S2b, S2c; Supplementary Table S7). When removing *pmoA* from the best model, the variance explained substantially decreases to 15% (Table 2b). Among all tested predictors, inorganic N and *pmoA* were the most important factors explaining CH_4 fluxes with WFPS and pH having the least importance in the models obtained (Fig. 4).

In the case of CO_2 emissions, the best and most parsimonious models contained three and two predictor variables. Water-filled pore space, soil temperature and bacterial (16S *rRNA*) abundance explaining 65% of the variance found in CO_2 emissions and WFPS and soil temperature alone explaining 61% (Table 2c). However, when considering 16S *rRNA* gene abundance as the unique driver of CO_2 fluxes, this predictor explains 30% of the variance (Table 2c) and a significant positive relationship was still found in all treatments ($P < 0.001$; Supplementary Figure S3a, S3b, S3c; Supplementary Table S8). Water-filled pore space, soil temperature and 16S *rRNA* gene abundance were the predominant and equally important predictors of CO_2 emissions within the variables considered (Fig. 4).

4. Discussion

4.1. Quantification of N_2O, CH_4, and CO_2 emissions and net GWP under management practices

Our results show the soil from this study is not a natural source of N_2O since most fluxes measured in C treatment were negligible. In fact, only fertiliser (F and IF) amended treatments had significant N_2O emissions. These findings are supported by a meta-analysis study which shows that N amendment results in greater N_2O release from non-agricultural soils (Aronson and Allison, 2012). Surprisingly, I treatment alone did not affect N_2O emissions but F and IF treatments had significant N_2O fluxes, with IF having 16-fold higher fluxes compared to F. Consequently, forest management practices (F and IF) had significantly increased net soil GWP. All soils under C, F, I and IF acted as a CH_4 sink, with rates of CH_4 oxidation in forest soils known to generally exceed those of other ecosystems and land-use (Dalal and Allen, 2008). However, under N fertilised amendment with no irrigation (F), the CH_4 sink was substantially reduced which directly increased its contribution to the soil net GWP. Under the remaining treatments, the CH_4 sink made no contribution to the net GWP when considering N_2O, CH_4, and CO_2 altogether. This is because CO_2 is the main contributor to net GWP due to its higher soil efflux in comparison to N_2O and CH_4. Carbon dioxide emissions (soil respiration) were substantially increased in I and IF whereas a significant decrease under F was observed. Previously, a reduction of soil microbial respiration due to N fertilisation has been reported which was attributed to shift in metabolic capabilities or soil microbial communities (Bowden et al., 2004; Ramirez et al., 2010) Our results also suggest that in this ecosystem, microbial respiration was further constrained by water availability. This reduction in soil respiration leads to a change of direction of the net GWP of soils under F treatment. This contrasts with CO_2 contribution to a higher net GWP from soils under I and IF. Nonetheless, this study does not take into account the CO_2 emission balance between aboveground vegetation and belowground respiration. In fact, higher CO_2 emissions found in I and IF are also followed by higher tree height and diameter (Frew et al., 2013) which will compensate the CO_2 efflux from soil and hence reduce the net GWP of the managed plantation studied.

4.2. Impact of management practices and environmental drivers on N_2O emissions

Our results suggest that ammonia-oxidizers, through nitrification-mediated processes are the principal source of N_2O emissions in the IF treatment, since soil NH_4^+ appears to be the substrate for N_2O emissions as the significantly higher emissions coincided with a significant decline in NH_4^+. Nitrous oxide production in soil by autotrophic nitrification is traditionally considered to be minor in comparison to heterotrophic denitrification (Dalal and Allen, 2008). However nitrification, rather than denitrification have been reported to be the main source of N_2O

Table 2
Best-fitting regression models of N_2O, CH_4 and CO_2 fluxes. Each column represents a different predictor variable with corresponding colour (WFPS, soil temperature (for CO_2), inorganic N, pH and microbial gene abundances). The best 8 models are presented, ranked according to AIC_c value. The last models show when microbial predictors and/or abiotic predictors are removed from the best model obtained. Unshaded cells indicate variables that were not included in a particular model. From the best 8 models selected: a) for N_2O, of all 63 possible models the first and sixth are the best and most parsimonious respectively; b) for CH_4 of all 15 possible models the first and the seventh are the best and most parsimonious respectively; c) for CO_2 of all 31 possible models the first and the sixth are the best and most parsimonious, respectively. ΔAIC_c = difference between the AIC_c of each model and that of the best model; AIC_c w_i = Akaike weights; WFPS = water-filled pore space; AOA = ammonia-oxidizing archaea, AOB = ammonia-oxidizing bacteria.

Table 2a

WFPS	Inorganic N	pH	AOA	AOB	*nosZ*	R^2	AIC_c	Δ AIC_c	AIC_c w_i
						0.248	17.067	0	0.262
						0.243	18.268	1.201	0.144
						0.250	18.674	1.607	0.117
						0.249	18.950	1.883	0.102
						0.248	19.021	1.954	0.099
						0.227	20.121	3.054	0.057
						0.251	20.548	3.481	0.046
						0.225	20.574	3.506	0.045
						0.120	40.742	23.675	<0.001
						0.109	43.285	26.217	<0.001
						0.011	63.255	46.187	<0.001

Table 2b

WFPS	Inorganic N	pH	*pmoA*	R^2	AIC_c	Δ AIC_c	AIC_c w_i
				0.357	1392.016	0	0.479
				0.359	1393.546	1.530	0.223
				0.357	1394.109	2.093	0.168
				0.361	1394.912	2.896	0.113
				0.336	1400.186	8.171	0.008
				0.324	1401.503	9.487	0.004
				0.316	1401.774	9.759	0.004
				0.316	1403.848	11.833	0.001
				0.150	1443.428	51.412	<0.001

Table 2c

WFPS	Soil temperature	Inorganic N	pH	16S *rRNA*	R^2	AIC_c	Δ AIC_c	AIC_c w_i
					0.647	-249.835	0	0.486
					0.648	-248.189	1.646	0.213
					0.648	-247.958	1.877	0.190
					0.650	-246.861	2.974	0.110
					0.613	-232.145	17.690	<0.001
					0.608	-231.512	18.323	<0.001
					0.613	-230.072	19.764	<0.001
					0.608	-229.754	20.081	<0.001
					0.304	-123.408	126.427	<0.001

emissions in semi-arid regions as soils are rarely sufficiently anaerobic to induce denitrification (Barton et al., 2008; Galbally et al., 2008). Furthermore, the capability to denitrify by AOA and AOB makes nitrifier denitrification a distinct pathway from denitrification because it is not negatively impacted by oxygen. In addition, it was found that N_2O emissions under IF were positively correlated with *amoA* gene abundance of AOA and AOB as well as with WFPS but not with *nosZ* gene abundance. This provides support for the argument that ammonia-oxidizers play a significant role in N_2O emissions under increased water and N availability. In fact, Hu et al. (2015), reported significantly higher potential nitrification rates and *amoA* activity under the same treatment when studying the metabolic activity of ammonia oxidizers in the same field site. Nonetheless, under F, even though N_2O emissions were significantly higher in comparison to C, they were much lower than IF. Ammonium, NO_3^- and PO_4^{3-} were also found to be three, eight and seven times higher in F in comparison to C, suggesting a reduction in the uptake of these nutrients by plants and/or microbial communities, possibly due to water limitation of microbial activity. Plots without irrigation treatments (C and F) were clearly

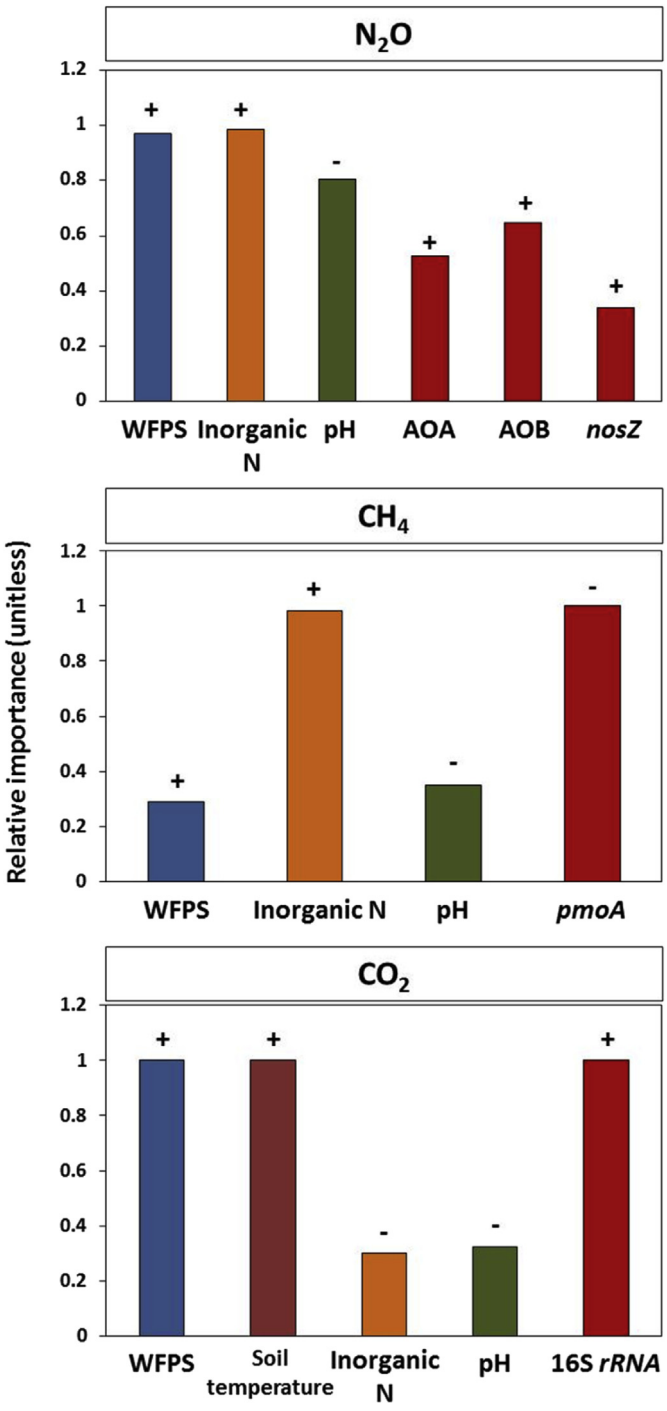


Fig. 4. Relative importance of environmental drivers such as microbial gene abundances (red columns) and other abiotic properties as predictor variables in models of N_2O, CH_4 and CO_2 emissions. The height of each column is the sum of Akaike weights (w_i) of all models that included the predictor of interest, taking into account the number of models in which each predictor appears. Positive (+) and negative (−) signals on top of each column corresponds to the direction of estimates for each predictor variable. Variable abbreviations are as in Table 2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

water limited with WFPS and gravimetric moisture content values of approximately 5% and 2%, respectively. Together with flux data, our results provide evidence that both N_2O emissions and functional microbial communities were water and substrate limited in this ecosystem. Indeed, Hu et al. (2015) found similar results and showed that AOA and AOB communities remained inactive or dormant in the treatments without irrigation (C and F) in comparison to irrigated treatments (I and IF).

In our study a significant reduction of *nosZ* gene abundance was also observed under F. It has been found that denitrification enzymes can remain active under aerobic conditions, with the exception of N_2O reductase, which seems to be more sensitive to O_2 (Morley et al., 2008; Richardson et al., 2009). Additionally, low pH is also known to slow down turnover and assembly of N_2O reductase (Richardson et al., 2009; Bergaust et al., 2010), with reports of increasing N_2O:N_2 ratio with decreasing soil pH (Šimek et al., 2002; Dannenmann et al., 2008; Barton et al., 2013).

In an attempt to further identify environmental drivers that best explain N_2O emissions in a dryland forest, a multi-model inference approach was applied. The best model describing N_2O emissions from all management practices studied explained 25% of the variance found in N_2O fluxes and included WFPS, inorganic N, pH as well as AOB. The low variance explained highlights that complex pathways responsible for N_2O emissions are not fully accounted for. All microbial groups were positively related to N_2O, with higher importance of ammonia oxidizers over N_2O-reducing bacteria abundance. Nonetheless, this study is limited to gene abundance and does not take into account other factors, including gene expression (Braker et al., 2012) which could help determine whether N_2O-reductase remained inactive, particularly in F. Overall, we provide evidence that N_2O emissions are directly linked to water availability, substrate concentration and functional microbial communities. Our results further suggest that in dryland forest ecosystems, nitrifier-mediated processes, nitrification and/or nitrifier denitrification, are important pathways for N_2O emissions.

4.3. Impact of management practices and environmental drivers on CH_4 emissions

Methane fluxes observed in this study were negatively correlated with *pmoA* gene abundance in all treatments with the strongest relationship found in F. Furthermore, both CH_4 uptake and *pmoA* gene abundance were significantly reduced under F. The effect of N fertilisers on CH_4 fluxes have been widely studied in recent years and it is thought that N can inhibit CH_4 uptake in soil due to competitive inhibition of CH_4 oxidation at the microbial enzyme level (Bodelier, 2011). This is because enzymes which carry out CH_4 oxidation and ammonia oxidation, have a similar structure and substrate specificities and therefore both compete for O_2, CH_4 and NH_3 (Mosier et al., 2004). This in turn can create an inhibitory effect of NH_4^+ produced in the soil and/or added through ammonia based fertilisers on CH_4 oxidation (Hanson and Hanson, 1996; Dalal et al., 2008). In fact, in the IF treatment, NH_4^+ concentration was much lower in comparison to F which could explain the higher CH_4 oxidation observed in this treatment, potentially leading to less inhibition of CH_4 oxidation. Furthermore, in F, both NH_4^+ and NO_3^- were negatively correlated to *pmoA* abundance as opposed to no significant correlation in the remaining treatments, providing evidence of the negative impact of N accumulation in the soil on CH_4 oxidation activity.

Taking a multi-model inference approach it has shown that 36% of the variance of CH_4 flux across all management practices was explained by inorganic N and *pmoA* abundance. When removing *pmoA* as a potential predictor of CH_4 flux from the best model obtained, the variance explained substantially decreased to 15% suggesting that the abundance of methanotrophs has a direct effect on the net CH_4 emission balance in these soils. Our finding is consistent with a previous report which linked methanotroph abundance with CH_4 oxidation rates (Menyailo et al., 2008). In fact, inorganic N

and *pmoA* were the predictors with higher importance in all models generated, with inorganic N having a positive impact on CH_4 fluxes and all the remaining predictors having a negative impact on CH_4 fluxes (i.e. by increasing CH_4 oxidation). It could be expected that WFPS would play a more important role in predicting CH_4 fluxes by directly affecting soil O_2 levels and diffusion rates (Tate et al., 2007), but its low importance suggests the fluctuations observed between treatments were not sufficient enough to negatively alter CH_4 oxidation rates.

4.4. Impact of management practices and environmental drivers on CO_2 emissions

Our results show a significant increase in CO_2 flux under I and IF. Increased water availability in dryland ecosystems is known to increase soil respiration, particularly in the form of pulses of increased CO_2 emissions following rainfall events (Yan et al., 2011, 2014). We found a significant correlation between bacterial abundance and CO_2 fluxes in all treatments providing evidence that bacterial abundance has a substantial role in soil CO_2 emissions. Furthermore, bacteria are the most abundant microbes in the soil (Singh et al., 2009a), including at this site (Federica Colombo personal communication), making them the most important driver of soil CO_2 emissions (Singh et al., 2010). The reduction in soil respiration due to N fertilisation could be attributed to a decline in microbial biomass (Treseder, 2008) or to a reduction in organic matter decomposition, where N is not limiting microbial growth (Janssens et al., 2010). We not only observed a significant reduction of CO_2 emissions in F but also a significant reduction of bacterial abundance was evident in this treatment, with both CO_2 and 16S *rRNA* abundance showing a negative correlation with both NH_4^+ and NO_3^-. Furthermore, CO_2 emissions and 16S *rRNA* abundance were significantly increased in IF which suggests that the soil respiration response to N addition was highly dependent on water availability since water availability is known to moderate the effect of other factors on soil respiration (such as temperature and substrate supply; Yan et al., 2011). It is possible that increased root respiration had also contributed to increasing soil CO_2 emissions under IL treatment because increased tree growth was observed in this treatment.

Water-filled pore space, soil temperature and 16S *rRNA* abundance explained 65% of the variance of CO_2 emissions by applying a multi-model inference approach, whereas 16S *rRNA* abundance alone explained 30%. Furthermore, these three predictors were the most important ones in all models accounted for by increasing CO_2. Clearly soil temperature, together with moisture are paramount in controlling microbial activity (Singh et al., 2010) and thus CO_2 efflux from soil (Karhu et al., 2014). Hence, in this study both seem to be key environmental factors affecting microbial growth and activity. Inorganic N and pH were less important in predicting CO_2 emissions which further suggests that in dryland ecosystems even though N can reduce soil respiration this is highly dependent on the antecedent soil water availability and optimal temperature for microbial activity.

4.5. Impact of land-use change (afforestation) and environmental drivers on GHG emissions and net GWP

Land-use change, as in afforestation, did not significantly affect differences in soil N_2O emissions and CH_4 uptake, whereas a reduction in CO_2 emissions was observed. In fact, N_2O emissions were virtually non-existent under both G and C. Previous studies have reported that it may take more than 8 years before afforestation-mediated changes in GHG fluxes can be detected (Singh et al., 2009b; Nazaries et al., 2011). This may explain the lack of difference in GHG fluxes between G and C, because our forest plantation is only six years old. It was expected that there would be negligible contributions of both N_2O and CH_4 to net GWP in afforested soils, with the main contribution coming from the reduction of soil CO_2 emissions leading to a negative net GWP.

Gene abundances of ammonia-oxidizers, N_2O-reducing bacteria and methanotrophs were also not significantly different in G and C whereas 16S *rRNA* abundance was significantly different between treatments and positively correlated to CO_2 emissions. Although changes in multiple abiotic factors were observed due to afforestation (such as decrease in NH_4^+, pH and WFPS), these do not seem to be enough to substantially alter N_2O and CH_4 related microbial abundance. Studies have shown lower soil respiration in woodlands relative to grasslands (Raich and Tufekciogul, 2000; Smith and Johnson, 2004) and even a reduction from natural forests to plantations has been found (Sheng et al., 2010). Our results are similar, with a significant reduction of CO_2 emissions under afforested soils. Moreover, it has been shown that the conversion of grasslands to woodlands can have limited effects on soil N processes (McKinley et al., 2008) and our study shows reduced effect of afforestation on inorganic N. This suggests that other environmental drivers can be affecting soil respiration, not to mention the contribution of grass root respiration not accounted for in our measurements. Water-filled pore space and soil temperature were significantly decreased under afforestation and also positively correlated to CO_2, further supporting previous reports of water availability and soil temperature as a main driver of CO_2 emissions (Davidson et al., 2006).

4.6. Conclusions

We identify mechanistic pathways and drivers of GHG fluxes under a dry sub-humid ecosystem. The soils from the dryland forest ecosystem studied were not natural emitters of N_2O and CH_4 but under current management practices their individual contributions to net GWP increased. Overall, soil N_2O and CO_2 fluxes were limited by water whereas N_2O and CH_4 were further constrained by N availability. Nitrous oxide emissions showed a strong relationship with ammonia-oxidizing communities, suggesting that nitrifier-denitrification pathway could be a principal contributor to N_2O emissions. All soils were a CH_4 sink but the sink capacity was constrained by the addition of N fertilisers which was linked to the abundance of methane-oxidizing community. This study also provides novel evidence that functional microbial groups are the major predictors of GHG emissions along with water and substrate availability. Our findings improve the mechanistic understanding of GHG emissions in dryland forest ecosystems, which should be considered in formulating future mitigation options.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.03.012.

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