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Short communication

Nitrous oxide emissions following dairy shed effluent application beneath *Kunzea robusta* (Myrtaceae) trees

Hannah M. Franklin[a,c,*,1], Roshean R. Woods[b,1], Brett Robinson[b], Nicholas Dickinson[a]

[a] Department of Ecology, Lincoln University, PO Box 85084, Lincoln, 7647, Canterbury, New Zealand
[b] Department of Soil and Physical Sciences, Lincoln University, PO Box 85084, Lincoln, 7647, Canterbury, New Zealand
[c] Australian Rivers Institute, Nathan Campus, Griffith University, 170 Kessels Road Nathan, Queensland, 4111, Australia

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ABSTRACT

Agriculture contributes more than a third of anthropogenic nitrous oxide (N_2O) emissions globally. In New Zealand, land application of dairy shed effluent contributes to the 90% of N_2O emitted from agricultural soils. Novel strategies are urgently required to mitigate N_2O production to ensure New Zealand's dairy-based economy is environmentally sustainable. Species of Myrtaceae, including *Kunzea* spp. (kānuka, white tea-tree) have previously been shown to produce antimicrobial compounds which extend to the soil. It is possible that these may inhibit the microbes involved in biological nitrification and denitrification which could thereby suppress N_2O production. Therefore, in this work we aimed to test whether irrigation of effluents to stands of *Kunzea* spp. could minimize resulting N_2O emissions. This study investigated soil inorganic N and N_2O emissions following the application of dairy shed effluent to soil beneath 5-yr-old *K. robusta* compared with bare soil. Following effluent application, N_2O emissions beneath *K. robusta* were reduced by 80% relative to bare soil, but nitrate-N was five-fold higher than bare soil, sufficiently available for denitrification. The drier, more aerated soil associated with *K. robusta* may have constrained denitrification. Application of DSE (50 kg N ha^{-1}) to *K. robusta* produced 0.133 kg N_2O–N ha^{-1} during the experimental period; equivalent to the lower range of emissions measured following comparable applications to grazed dairy pastures in New Zealand (0.13-1.08 kg N ha^{-1}). The environmental benefits of reduced N_2O emissions warrant further investigation on the effect of Myrtaceae on the soil N cycle worldwide.

1. Introduction

Nitrous oxide (N_2O) is a potent greenhouse gas with a global warming potential 298 times that of carbon dioxide (Myhre et al., 2013). New Zealand has a unique greenhouse gas profile where 93.4% of N_2O emissions are from agricultural soils (Ministry for the Environment, 2015). Land irrigation of dairy shed effluent (DSE) to pastures contributes to these N_2O emissions and is commonplace in New Zealand. Dairy shed effluent is typically a high-nitrogen (N) mixture of urine, dung, and wash-down water containing 44-628 mg N L^{-1}; mostly in the form of organic N (Saggar et al., 2004).

Findings of a recent study suggest New Zealand members of the Myrtaceae family could mitigate the threat of microbial contamination of soil following the application of organic wastes (Prosser et al., 2016). The antimicrobial properties of *Leptospermum* (mānuka, tea-tree) and *Kunzea* (kānuka, white tea-tree) species were found to extend to the soil. However, this study did not consider soil N processes. Biological nitrification converts ammonium-N (NH_4^+) in DSE to nitrate-N (NO_3^-) in soil, which is the substrate for N_2O production via the nitrifier-denitrification and denitrification of anaerobic bacteria (Bolan et al., 2004). The antimicrobial properties of Myrtaceae could potentially influence these microbially driven soil N cycling processes and alter nitrate (NO_3^-) production, denitrification and N_2O emissions. In this study, we investigated the suitability of *Kunzea robusta* for the disposal of DSE in terms of N_2O production.

We hypothesized that application of DSE to *K. robusta* trees could reduce N_2O emissions compared with application to bare soil. The aim of the present study was to compare changes in N_2O emissions and soil inorganic N concentrations from soil underneath

* Corresponding author at: Australian Rivers Institute, Nathan Campus, Griffith University, 170 Kessels Road, Nathan, Queensland, 4111, Australia.
E-mail addresses: h.franklin@griffith.edu.au, hannahfranklin23@gmail.com (H.M. Franklin).
[1] These authors contributed equally to practical work and to preparation of this manuscript.

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Kunzea trees with bare soil areas following DSE application. We also compared N_2O emissions from *Kunzea* with emissions data from previous studies of DSE application to grazed pastures in New Zealand.

2. Materials and methods

2.1. Site description

Kunzea robusta is one of 10 species previously described as *Kunzea ericoides* (de Lange, 2014). The experimental site was a fenced, planted corner of the Lincoln University Dairy Farm, Canterbury, New Zealand that was retired from grazing in 2008 (1800 m^2, 43°38′38.07" S, 172°26′1.96" E, described in Franklin et al., 2015). The soil type was a Templeton silt loam (Immature Pallic, Hewitt, 1998; Udic Haplustept, Soil Survey Staff, 2014). *Kunzea robusta* trees were around 2 m tall and 5 years old, with a 5–10 mm litter layer.

2.2. Treatment and experimental design

On 26 July 2012, headspace chamber bases (0.48 m diam., 0.18 m^2, stainless steel) with an annular water trough were inserted 0.1 m into the soil (gas sampling plots, Fig. 1). During gas sampling events, insulated, stainless steel headspace covers created a gas-tight seal (~0.14 m above the soil surface). Adjacent to each gas sampling chamber a metal ring (0.48 m diam., 0.18 m^2) was inserted 0.1 m into the soil for destructive soil sampling (soil sampling plots, Fig. 1).

Five replicate *K. robusta* and bare soil locations (bare soil >1 m from the drip line of trees) were randomly selected. Each location consisted of two subplots to which DSE or control was applied (Fig. 1). Each subplot consisted of a pair of gas sampling/soil sampling plots (Fig. 1). Dairy shed effluent (450 mg N L^{-1}) was applied at 50 kg N ha^{-1} to DSE subplots and an equal volume of untreated tap water (1.5 mg NO_3^--N L^{-1} and 0.05 mg NH_4^+-N L^{-1}) to the control subplots. Plots were established radially around *K. robusta* stems (0.2 m from the stem) and centers of bare soil locations, separated by >0.2 m, to avoid seepage between plots (Fig. 1). Treatments were applied to subplots (gas sampling/soil sampling pairs) randomly on the morning of 7 Aug. 2012. Dairy shed effluent was collected from the Lincoln University Dairy Farm storage pond on 9 Sept. 2011, homogenized, and stored at 5 °C prior to use.

2.3. Field sampling, analysis, and meteorological measurements

Gas samples were taken the day prior to treatment application (Day 0), approximately 4 h after DSE application (Day 1), and on following Days: 2, 3, 9, 16 and 23. Sampling was conducted between 12:30 and 15:30 h. Headspace samples (10 mL) were taken manually 0, 20, and 40 min after positioning the cover and compressed into 6 mL Exetainer tubes (Labco Ltd., High Wycombe, UK). The headspace temperature was recorded in a representative chamber after initial checks found similarity between locations. Immediately prior to analysis, gas samples were brought to ambient pressure and N_2O concentrations were determined using a gas chromatograph (GC, SRI 8610; SRI Instruments, CA, USA) fitted with a ^{63}Ni electron capture detector (Gilson 222 XL; Gilson Inc., WI, USA) and calibrated using BOC α standards (BOC Scientific, New Zealand). Nitrous oxide fluxes were calculated following Hutchinson and Mosier (1981) after checking for linearity in N_2O concentration over time. Cumulative N_2O emissions were determined by integration.

Soil core samples (2.5 cm diam. × 7.5 cm depth) were taken to monitor soil gravimetric moisture content (θ_g), pH, and inorganic-N concentrations. On Days: 0, 2, 3, 9, 16, and 23 three soil cores were taken at random, homogenized and sieved (≤4 mm). A subsample was dried at 105 °C for 24 h to determine θ_g. Soil pH was measured on field moist soil (10:25 soil:water). Another 4 g subsample of field moist soil was shaken with 40 mL of 2 M KCl for 1 h, centrifuged (10 min at 2000 rpm) and then filtered (Whatman No. 41) (Blakemore et al., 1987). Extracts were analyzed for NH_4^+-N and NO_3^--N by flow injection analysis (FOSS FIAstar 5000 triple channel with SoFIA software version 1.30; Foss Tecator, Hoganas, Sweden). Total C and N were measured on air-dried (35 °C for 48 h), ground, and sieved (2 mm) soil using an Elementar Vario-Max CN Elemental Analyzer (Elementar GmbH, Hanau, Germany). Total organic C was measured using the loss on ignition method (Blakemore et al., 1987). Samples were also analyzed for Olsen P following Olsen et al. (1954).

Soil bulk density was measured in *K. robusta* and bare soil locations in August 2015, after the experimental period. There had been no stock access since the trial. Cores (5.4 cm diam. × 5 cm depth) were carefully pressed into the soil. These were oven-dried (>48 h at 105 °C) to give θ_g which was used to calculate bulk density (g cm^{-3}). Soil particle density was assumed to be 2.65 g cm^{-3}. Water filled pore space (WFPS) was calculated from these and θ_g from the experimental period (Linn and Doran, 1984).

2.4. Statistical analysis

Tests for normality showed the N_2O flux data were skewed so these were log transformed (Ln[flux]). Daily and cumulative N_2O flux data were analyzed using analysis of variance (ANOVA), with a split–split plot design, effectively a special case of repeated measures ANOVA. Within whole plots (the 10 sampling locations), treatments (DSE and control) were randomly assigned to split-plots (subplots each consisting of a pair of gas/soil sampling plots). Within split-plots, sampling occurred on multiple dates (split–split plots). Plot type (*K. robusta* and bare soil), treatment (DSE and control) and time (Day) were factors. Three-way interactions were removed as the interpretation of such effects is complex. Soil WFPS, pH, NO_3^--N, and NH_4^+-N were analyzed in the same manner. Daily data were compared using ANOVA ($p < 0.05$) when significant interactions with time occurred. Two-sample *t* tests compared background soil conditions between bare soil and *K. robusta*. Statistics were performed in R version 3.0.1 (R Development Core Team, 2013, Vienna, Austria, http://www.r-project.org/).

3. Results

3.1. Nitrous oxide fluxes

Prior to treatment application (Day 0) N_2O flux was similar between locations (Fig. 2a). *K. robusta* had significantly lower N_2O fluxes compared with bare soil following DSE application until after Day 9 (Fig. 2a). Plot type, treatment ($p < 0.01$) and sampling date ($p < 0.05$) significantly affected daily N_2O flux, with the treatment effect changing temporally (treatment x time, $p < 0.05$). Nitrous oxide flux from *K. robusta*-DSE plots rose significantly only on Day 1 ($p < 0.001$).

Cumulative N_2O fluxes were higher ($p < 0.001$) from the bare soil-DSE plots (676 g N_2O—N ha^{-1} or 1.35% of applied N) compared with the bare soil-control (152 g N_2O–N ha^{-1}), *K. robusta*-DSE (133 g N_2O-N ha^{-1} or 0.27% of applied N) and *K. robusta*-control plots (95 g N_2O–N ha^{-1}), which were similar. Plot type ($p < 0.01$), treatment ($p < 0.05$), time ($p < 0.001$), and the plot type x treatment interaction ($p < 0.01$) were significant.

3.2. Soil and meteorological conditions

Background soil pH, total N, θ_g and WFPS were higher in bare soil locations, while total organic C and NO_3^--N were more concentrated beneath *K. robusta* (Table 1). Soil bulk density, total C,

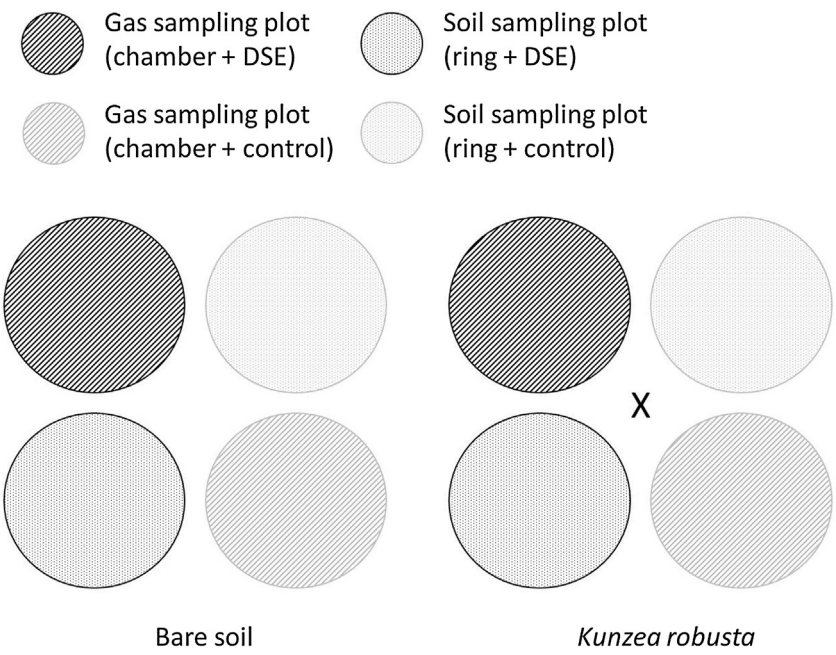

Fig. 1. The arrangement of dairy shed effluent (DSE) and control subplots in bare soil and *K. robusta* locations. Each subplot includes a paired gas sampling chamber and soil sampling plot (each 0.48 m diam.) to which DSE or control treatments were applied. Sampling plots were placed 0.2 m away from the stems of K. robusta (X) and centre of bare soil plots.

Table 1
Chemical and physical properties for the Templeton silt loam soil at the study site under *Kunzea robusta* and bare soil locations and of the dairy shed effluent. Data are mean (±SE). Soil (0–7.5 cm depth) was collected from *K. robusta* and bare soil locations adjacent to sampling plots (n = 5, except pH, NO_3^--N, NH_4^+-N and gravimetric moisture content where n = 10). Significant differences between *K. robusta* and bare soil locations are indicated.

	Bare Soil		*Kunzea robusta* (kānuka)		Dairy shed effluent
pH	5.5	(0.04)[b]	5.3	(0.05)	7.5
C (mg g^{-1})	29.9	(0.47) NS[d]	28.5	(0.60)	1070 (95)[c]
N (mg g^{-1})	2.94	(0.04)[a]	2.68	(0.09)	450 (45)[c]
Organic C (mg g^{-1})	5.99	(0.85)[a]	7.78	(0.17)	
C:N	10.2	(0.11) NS	10.7	(0.33)	
NO_3^--N (μg g^{-1})	7.04	(3.28)[a]	17.4	(4.03)	0.25 (0.01)
NH_4^+-N (μg g^{-1})	0.70	(0.53) NS[d]	1.32	(0.91)	244.9 (6.00)
Olsen P (mg L^{-1})	19.0	(1.21) NS	17.8	(1.67)	
Gravimetric moisture content (%)	31.4	(0.74)[a]	28.9	(0.64)	
Bulk density (g cm^{-3})	1.11	(0.03) NS	1.01	(0.04)	
Water filled pore space (%)	59.6	(1.40)[b]	46.9	(1.04)	

[a] Significant at the 0.05 probability level.
[b] Significant at the 0.001 probability level.
[c] Units are μg^{-1} for C and N of dairy shed effluent.
[d] NS, nonsignificant.

Olsen-P and NH_4^+-N did not differ between *K. robusta* and bare soil (Table 1).

K. robusta plots had significantly lower ($p < 0.001$) WFPS than the bare soil plots (Fig. 2b), however, WFPS did not vary due to treatment (DSE or control) or time. Mean daily θ_g ranged from 27 to 32% for *K. robusta* and 31- 35% for bare soil plots. A total of 104 mm of rain fell during the experiment, consisting of one substantial event (75 mm, Day 8–10, Fig. 2b). The mean daily air temperature ranged from 6.7 to 14.9 °C (Fig. 2b) and a spike later in the experiment corresponded to a decrease in soil moisture. Throughout the experiment soil pH was higher ($p < 0.05$) in the bare soil (mean of 5.8 ± 0.02) than *K. robusta* plots (5.5 ± 0.03), while treatment (DSE or control) and time did not affect pH.

Soil NO_3^--N concentrations were higher ($p < 0.01$) in the *K. robusta* plots than bare soil (Fig. 3a) and were not affected by treatment or time. Ammonium-N concentrations were significantly higher ($p < 0.05$) in DSE than control plots, but did not differ between *K. robusta* and bare soil (Fig. 3b). Ammonium-N was similar in all plots at the start of the experiment (Fig. 3b) and increased following treatment application ($p < 0.001$), but more so in DSE plots (treatment x time, $p < 0.01$). Ammonium-N spiked in control plots on Day 16 to match levels in DSE plots.

4. Discussion

4.1. Low nitrous oxide emissions under K. robusta

Cumulative N_2O emissions following effluent application were 80% lower beneath *K. robusta* relative to the bare soil. No other studies have investigated N_2O emissions from soil planted with *K. robusta* receiving DSE. Mean N_2O emissions from *K. robusta* locations (0.095 and 0.133 kg N ha^{-1} for control and DSE respectively) during the experimental period were close to background rates reported under *Kunzea* spp. elsewhere (below detection limits, Price et al., 2010; and 0.30 kg N ha^{-1} yr^{-1}, Hedley et al., 2013). Nitrous oxide emissions following DSE application to *K. robusta* dur-

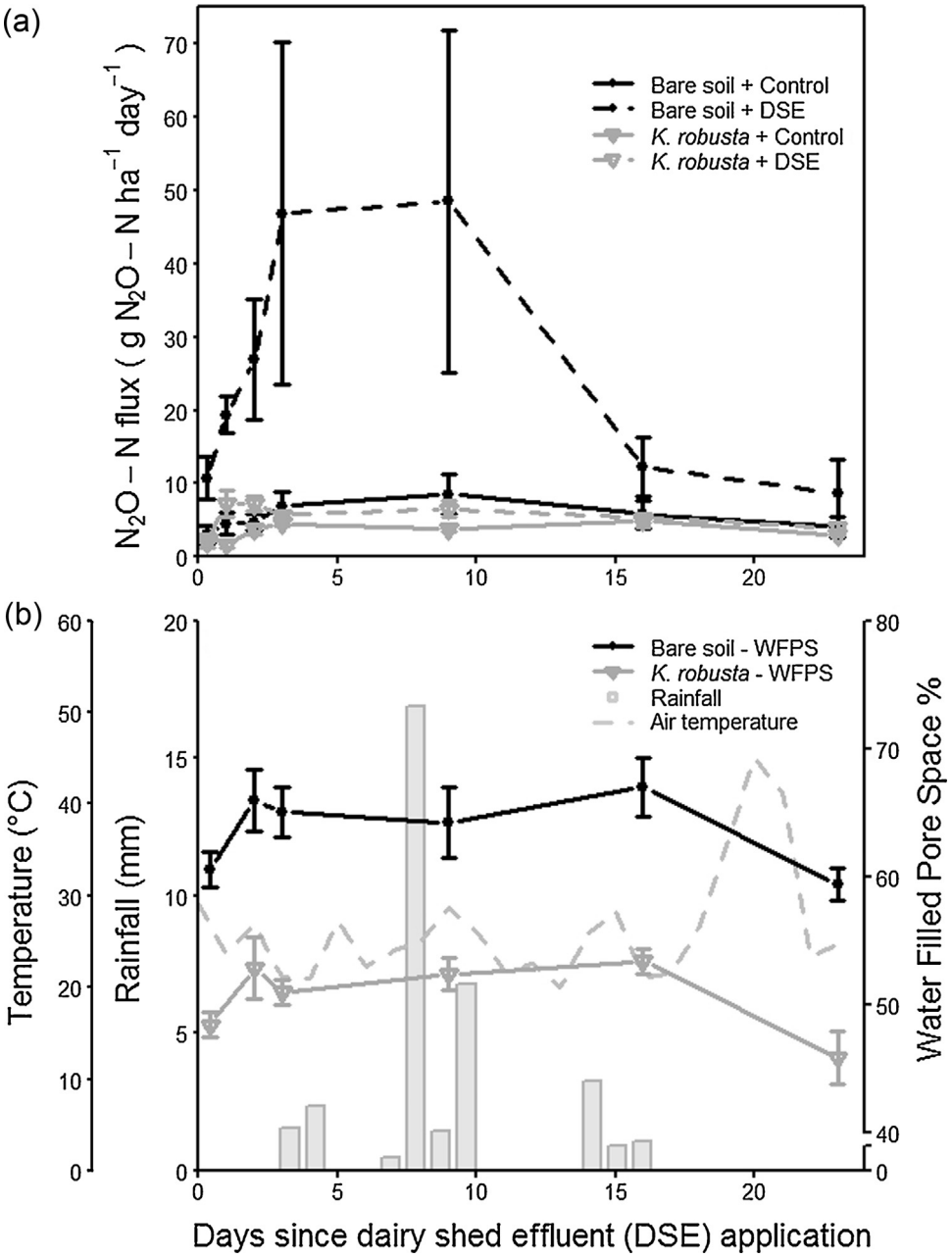

Fig. 2. Back-transformed mean (±SE) daily N_2O–N flux (a) and mean (±SE) water filled pore space and meteorological measurements (b) following the application of dairy shed effluent (DSE, 50 kg N ha^{-1}) and control treatments to *K. robusta* and bare soil plots (n = 5). Meteorological data were obtained from the nearby NIWA Broadfield Climate Station (43°37′34.4" S, 172°28′13.4 E). Water filled pore space data are for control plots only, dairy shed effluent plots not shown (no effect of treatment type).

ing the 24-day experimental period (0.133 kg N_2O–N ha^{-1}, 0.27% of N applied) were at the low end of those found in the literature for similar DSE applications to grazed pastures in New Zealand (0.13-1.08 kg N ha^{-1}). Following applications of 50–60 kg N ha^{-1} DSE N_2O was emitted at rates of 0.13-0.48 (0.25-0.8% of N applied, Li et al., 2015) and 0.15-0.45 kg N ha^{-1} (0.31-0.73% of N applied, Bhandral et al., 2007) during approximately 20 and 2 week experimental periods, respectively. While consecutive annual DSE applications of 56 and 43 kg N ha^{-1} in the Canterbury region produced 0.665 and 1.08 kg N_2O-N ha^{-1} yr^{-1} (1.2 and 2.5% of N applied, van der Weerden et al., 2016). Consistent with the current study, others also report N_2O emissions returning to background levels rapidly (< 3 weeks) following effluent application (Barton and Schipper 2001; Bhandral et al., 2010).

4.2. Soil conditions and nitrous oxide emissions

Water filled pore space was around 10% higher in bare soil than beneath *K. robusta*, likely due to transpiration by *K. robusta* and canopy rainfall interception. This was within the range suitable for denitrification (60–65%, Linn and Doran, 1984), however, WFPS in *K. robusta* plots were below this range. The corresponding aerobic conditions may have decreased N_2O-reductase activity in denitrification (Bakken and Dörsch, 2007) and potentially contributed to reduced emissions. Soil moisture has previously been associated with variation in N_2O emissions from *Kunzea* forests (Hedley et al., 2013). The dry sites which *Kunzea* spp. often dominate may explain the low reported background N_2O production rates (Hedley et al., 2013; Price et al., 2010). The control treatment

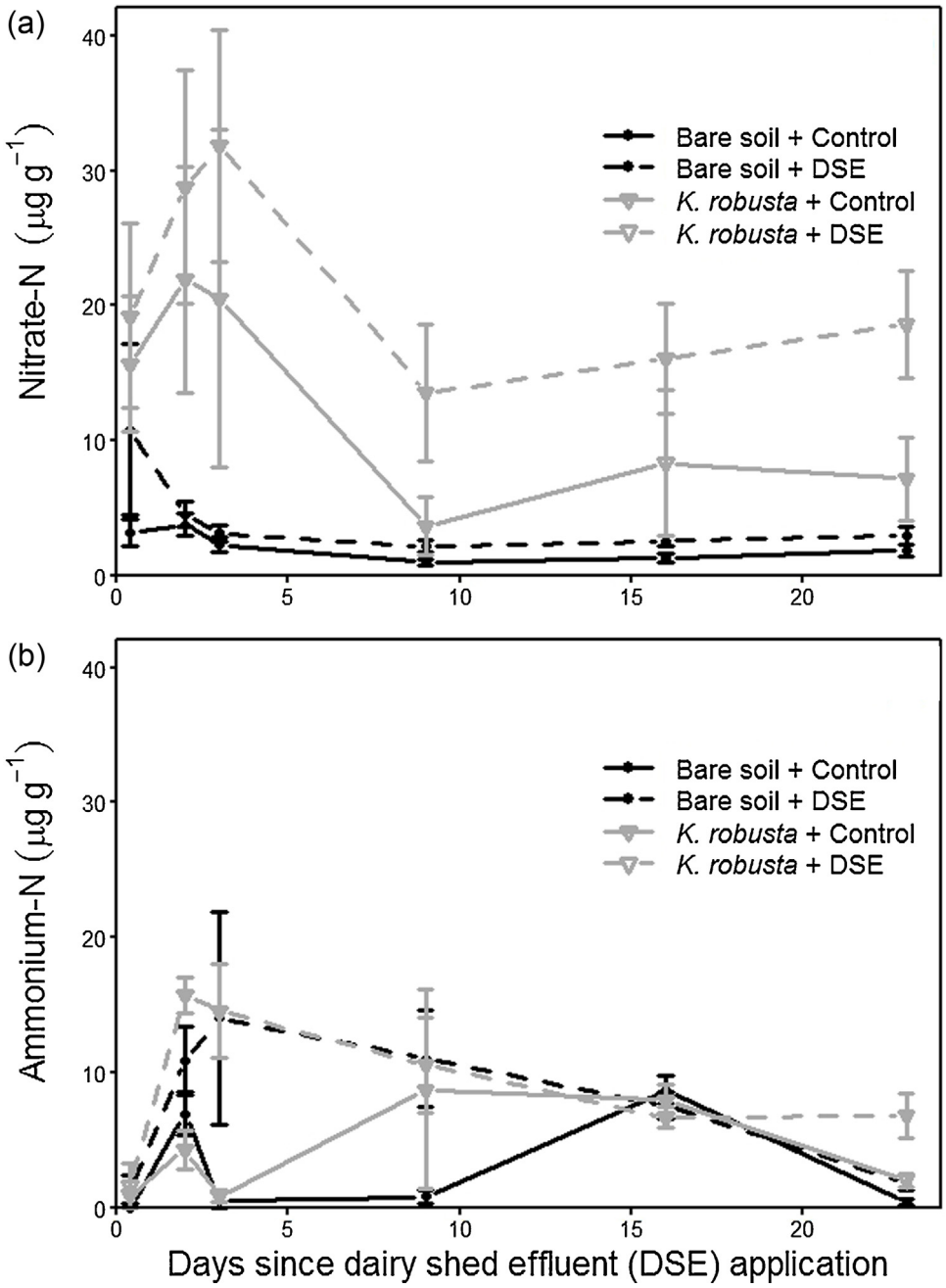

Fig. 3. Mean (±SE) soil NO_3^--N (a) and NH_4^+-N (b) concentration (μg^1 of soil, 0–7.5 cm depth) over time, following the application of dairy shed effluent (DSE, 50 kg N ha^{-1}) and control to bare soil and *K. robusta* plots (n = 5).

effectively accounted for increased soil moisture resulting from the DSE addition. However, WFPS did not increase in response to an event totaling 10% annual rainfall, it is possible that surface cracks channeled rainfall to groundwater or sampling dates missed a peak.

Soil pH was around 0.3 units lower beneath *K. robusta*, potentially through exudation of organic acids by *K. robusta* roots or breakdown of leaf litter. Denitrification rates are reduced in acidic conditions (van der Weerden et al., 1999), providing another possible explanation for reduced emissions. *Kunzea robusta* leaf litter at this site had a C:N ratio of 26 (Zhong et al., 2014) so some mineralization was likely, as reflected in the elevated soil organic C (Table 1).

Ammonium-N increased 10-fold immediately following DSE application likely due to the high NH_4^+ concentrations in the DSE. Plant uptake, microbial immobilization, or oxidization to NO_3^- may have then reduced the NH_4^+ pool over time. Loss of NH_4^+ through volatilization, is unlikely due to the low soil pH (Bolan et al., 2004). Ammonium-N also increased following water treatment possibly due to mineralization of soil organic matter, while rainfall may explain NH_4^+-N variability during the experiment. The peak in soil NO_3^--N following treatment application to *K. robusta* may be a result of increased NO_3^- retention. The rainfall event reduced soil NO_3^--N concentrations beneath *K. robusta* to levels comparable to bare soil.

The comparatively higher NO_3^--N under *K. robusta* may mean that the reduced N_2O emissions were not the result of inhibition of nitrifying bacteria, but rather inhibition of denitrifying bacteria. Despite available NO_3^- and C substrate for denitrification, *K. robusta* soil emitted less N_2O than bare soil. It seems likely that the dry, aerated, and acidic soil under *K. robusta* was less suitable for

N_2O production via denitrification. Differences in WFPS between *K. robusta* and bare soil confound the interpretation, meaning we cannot conclude whether inhibition of nitrification or denitrification occurred.

4.3. Implications for future myrtaceae research

We identified a significant reduction in N_2O production from soil beneath *K. robusta* following application of DSE, compared with bare soil. Constraints on the denitrification process in the dry *K. robusta* soil are a possible cause of reduced emissions (Hedley et al., 2013; Price et al., 2010). Nitrous oxide emissions from *K. robusta* were low compared to those following similar applications to dairy pastures, which typically have higher WFPS than that measured beneath *K. robusta* (Bhandral et al., 2007; Li et al., 2015). Irrigation of DSE onto *K. robusta* plantations may offer a sustainable disposal option for this high N waste, resulting in low N_2O emissions, even in the winter when emissions are typically higher. This would require a closed canopy prior to DSE application to minimize exposed bare soil which is likely to emit more N_2O. *Kunzea* plantations may also provide additional income to farms through the production of high value tea-tree oils and honey (Stephens et al., 2010). Other Myrtaceae, *Eucalyptus* (Tzanakakis et al., 2009) and *Melaleuca* spp. (Bolton and Greenway, 1999), have been successfully used in effluent land treatment systems to mitigate NO_3^- leaching and produce harvestable biomass, but associated N_2O emissions have not been investigated. Although suppression of denitrification is desirable, pollution swapping may occur through increased NO_3^- leaching. *Kunzea robusta* seedlings have demonstrated a capacity for luxury N uptake (Franklin et al., 2015). Nitrogen storage in *K. robusta* roots, wood and foliage may reduce the pool of soil nitrate available for both denitrification to N_2O, and NO_3^- leaching. Further research should simultaneously measure N_2O emissions, NO_3^- leaching and N uptake by *K. robusta*, to determine the net environmental effect of DSE irrigation onto *K. robusta*. In addition, future work should incorporate comparisons to other tree and grassland species to determine if this effect is unique to *K. robusta*. The environmental benefits of reduced N_2O emissions warrant a closer look at the effect of Myrtaceae on the soil N cycle worldwide.

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