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The potential of *L. scoparium*, *K. robusta* and *P. radiata* to mitigate N-losses in silvopastural systems☆

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ABSTRACT

Silvopastoral systems aim to enhance economic, cultural and social principles by sustainably combining forest management with agriculture. In these typically high-nitrogen (N) environments, plant species selection can profoundly influence N fluxes. For grazed pastures, plants may be exposed to urine patches that have received the equivalent of up to 1000 kg N ha^{-1}. We aimed to determine the growth and N fluxes in three potential trees that may be used in silvopastoral systems: *L. scoparium*, *K. robusta* and *P. radiata*. Plants were grown in a greenhouse lysimeter experiment, with controlled irrigation and temperature and exposed to N at rates of 200 kg ha^{-1} equiv. for 15 weeks, followed by the addition of 800 kg ha^{-1} N equiv, to simulate a urine patch. Urea produced a positive growth response of all plant species. Treatments containing *L. scoparium* and *K. robusta* leached lower amounts of nitrate (NO_3^-) (2 kg ha^{-1} NO_3^-) compared to *P. radiata* (53 kg ha^{-1}). Measurements of N_2O over 20 days after the application of 800 kg N ha^{-1} indicated an inhibitory effect of *L. scoparium* and *K. robusta* on denitrification, hence loss of N via N_2O. Both *L. scoparium* and *K. robusta* demonstrated that they have potential to reduce N-losses in silvopastural systems, while producing valuable biomass.

1. Introduction

Silvopastoral systems combine forestry and animal production, with trees grown in combination with forage plants and livestock, in an attempt to produce economic returns while minimizing negative impacts on the environment (López-Díaz et al., 2009). Revenue can be generated from plant products, such as timber (lumber) essential oils and honey, as well as livestock products. Silvopastoral systems may contribute towards sustainable agriculture, and can enhance biodiversity, nutrient cycling efficiency, animal welfare and income (Mosquera-Losada et al., 2005). However, interactions among trees, pasture and animals may pose management difficulties (Eichhorn et al., 2006).

Trees may compete with pasture for water, nutrients, and above all, light. Therefore species selection and fertilization strategies are critical in optimizing silvopastoral systems (López-Díaz et al., 2009). After successful plant establishment, fertilization is required to maintain productivity. Urea is the most used N fertilizer in many regions. Following hydrolysis, the ammonium released from urea rapidly nitrifies to nitrate, which can in turn leach or be denitrified and released as nitrogen or nitrous oxide, a potent greenhouse gas (Robertson and Groffman, 2015). Investigations of soil mineral N dynamics show NO_3-N concentrations in grazed pastures are 5–10 times higher than NH_4-N, indicating these systems are a high nitrifying environment with a high potential for NO_3^- leaching (Chang et al., 2002; Poudel et al., 2002). In grazed pastures animal urine adds N to the soil at rates of up to 1000 kg N ha^{-1} (Selbie et al., 2015). This N is far in excess of what can be taken up by pasture and consequently, urine patches are responsible for up to 70% of NO_3^- leaching in grazed pastures (Cuttle and Bourne, 1993). Trees can reduce NO_3^- leaching due to their greater effective root depth compared to pasture, which in turn can potentially increase fertilization use efficiency in silvopastoral systems (Mosquera-Losada et al., 2006). Moreover, animals may spend

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longer under trees that provide shade and shelter (Mosquera-Losada et al., 2005), thereby depositing a disproportionate amount of urine onto the trees' root zones.

Nitrification rates may be affected by both trees and herbaceous species. Plants may enhance or inhibit nitrification, via root exudates (Subbarao et al., 2009) or compounds released into the soil from abscised leaves (Northup et al., 1995; Paavolainen et al., 1998). Such biological nitrification inhibition (BNI) occurs due to the inhibition of enzymes that are involved in the nitrification process (Subbarao et al., 2013), or by changing microbial communities in soil (Amaral et al., 1998). Nitrification inhibiting properties have been reported for polyphenolic compounds (Castaldi et al., 2009; Rice and Pancholy, 1973) or monoterpenes (White, 1988), which are commonly found in pine litter (Northup et al., 1995). *P. radiata* stands have been observed to inhibit nitrification in a New Zealand field site (Cooper, 1986), and leaf extracts decreased nitrification in soil incubation trials (Suescun et al., 2012). Inhibiting nitrification subsequently affects denitrification, since less NO_3^- is available as substrate for N_2O production. Plant species with BNI properties can potentially reduce N_2O losses associated with nitrification (Subbarao et al., 2013). Monterey pine (*Pinus radiata* D. Don) is one of the world's commercially dominant tree species, and is well integrated in silvopastoral systems in temperate areas such as Australia, New Zealand and Chile (López-Díaz et al., 2009). However, decreases in log prices have reduced the incentive to replant harvested trees. In New Zealand and elsewhere, there is increasing interest in incorporating native species into farming systems to improve the quality of surface waters (Franklin et al., 2015). Mānuka (*Leptospermum scoparium* J.R. Forst & G. Forst) and kānuka (*Kunzea robusta* de Lange & Toelken) are in the myrtaceae family and are common throughout New Zealand and Australia (Bergin et al., 1995; Stephens et al., 2005). Both, mānuka and kānuka are attracting interest due to their role in the production of essential oils (Maddocks-Jennings et al., 2005; Song et al., 2013) and honey (Beitlich et al., 2014; Steinhorn et al., 2011). Compounds associated with mānuka and kānuka have demonstrable antimicrobial properties (Prosser, 2011; Wyatt et al., 2005). Prosser et al. (2016) found that mānuka killed *E. coli* in soil. We hypothesise that these antimicrobial compounds associated with these species may affect N cycling, which is microbially mediated and therefore reduce N leaching or gaseous N emissions (Castaldi et al., 2009; White, 1988).

Mānuka and kānuka are adapted to low fertility environments, hence their growth responses to N resulting from fertilization or animal excreta in agricultural systems are unknown (Franklin et al., 2015), whereas pine responds positively to N (Neilsen et al., 1992). Increasing biomass production by fertilization while simultaneously minimizing N losses from these systems could enhance the sustainability of silvopastoral systems, while increasing productivity and revenue due to the high-grade timber and plant related products, such as honey and essential oils (Porter and Wilkins, 1999; Stephens et al., 2010).

We aimed to determine whether urea could increase the biomass production of mānuka and kānuka and the potential of these species to reduce N_2O-emissions and NO_3^- leaching in silvopastoral systems. We sought to compare these with *Pinus radiata*, which is common in silvopastoral systems, responds positively to N, and has allochemicals that inhibit nitrification.

2. Material and methods

In May 2013, 10 L lysimeters were constructed and installed at the Lincoln University plant growth facility (43°38′42″S 172°27′41″E). Each lysimeter was filled with 10 L of soil at an average soil bulk density of 1.0 g cm^{-3}3. The soil was collected from a marginal slope of the North Island New Zealand near Bideford (40°45′56″S 175°54′42″E), which was representative of many low fertility soils under native vegetation, dominated by mānuka (*L. scoparium*) and kānuka (*K. robusta*). Soil analyses showed the soil was pH 6.1, with a medium carbon (6.5%) and N (0.46%) level, a C/N ratio of 14.3 and a low level of bicarbonate (Olsen) extractable phosphorus (11 mg L^{-1}). Cation exchange capacity (CEC) was determined at 21 me 100 g^{-1}. To measure NO_3^- leaching, a leachate-sampling device was installed in the bottom of each lysimeter, covered by fleece sheets and a gravel drainage layer to avoid stagnant moisture.

Two native New Zealand trees, mānuka and kānuka, as well as pine, were grown with or without urea fertilizer, replicated four times. All species were planted into the lysimeters on the 6th of August 2013, after they had been filled and settled for eight weeks. *P. radiata* seedlings (GF19) were purchased from Southern Woods Nursery Ltd, Christchurch/New Zealand. *L. scoparium* (PH lebsco, 6M) and *K. robusta* (KV kunert, 6M) were purchased from Wai-ora Nursery Ltd, Christchurch/New Zealand. All seedlings were purchased in rootrainers at a seedling size of between 35 and 50 cm. To allow N_2O measurements, a chamber base for gas sampling (10 cm in diameter and 10 cm in height) was installed (5 cm deep) into each lysimeter. After all plants have been established in the lysimeters for six weeks, urea was applied onto the lysimeters. The urea treatments received an equivalent of 200 kg N ha^{-1} in total, which is the maximum allowable annual rate that N can be applied in most NZ jurisdictions (Pyke et al., 2016). The urea application occurred as four 0.53 g applications per lysimeter, equivalent to 50 kg ha^{-1} each application on September 16th, October 7th, October 29th and November 18th of 2013. Control treatments did not receive any urea.

Prior to final harvest, a urea application of 8.48 g of urea (800 kg N ha^{-1} equiv.) was carried out, simulating a ruminant urine event commonly occurring in farm systems (Oenema et al., 1997; Selbie et al., 2015). This high N application was followed by N_2O measurements for 20 days. For N_2O measurement, a polypropylene chamber was placed over the permanently installed ring for 40 min and sealed with a worm drive hose clip, resulting in a headspace volume of 500 mL. Samples were taken immediately after chamber placement (t0), after 20 (t1) as well as 40 min (t2) with a syringe and needle inserted at a rubber septum on top of the chamber. The greenhouse temperature during the time of N_2O measurement was recorded for flux calculations (Fig. S1 - Supplemental material).

Watering during the experimental period was carried out via an automated irrigation system, which allowed an independent watering of each plant species by pressure compensated drippers. Irrigation was controlled and adjusted daily to provide at ca. of 20–30 mm of drainage per week to account for differing rates of evapotranspiration and near-field capacity conditions.

A final destructive harvest of all lysimeters was performed after 20 days of N_2O measurement. The above-ground and below-ground plant biomass were weighed and oven-dried at 60 °C until a constant weight, carefully separated and reweighed. Soil attached to the roots ≤2 mm was considered as rhizosphere soil. Rhizosphere soil was harvested, subsampled and stored for further analyses at 4 °C after sieving ≤5 mm.

2.1. Analyses and measurements

Soil pH was measured from rhizosphere soil samples by adding 25 mL of DI water to 15 g of fresh soil (Blakemore et al., 1987). Total N in plant and soil material was analysed using oven dried (60 °C) and ground material using a CNS-2000 Element Analyser (LECO Australia Pty Ltd, Australia). In order to investigate the influence of

the plant species on N transformation and translocation into the soil, the rhizosphere soil was used to determine the soil inorganic N speciation. A KCl extraction was carried out from fresh soil (4 °C) within 4 days after harvest according to (Blakemore et al., 1987). After adding 40 mL of a 2M KCl reagent to 4 g of soil, the solution was shaken on an end-over-end shaker for 1 h, centrifuged at 827 g for 10 min and subsequently filtered through Whatman 41 filter paper. Nitrate-N (NO_3-N) and ammonium-N (NH_4-N) were determined using a flow injection analyser (FIA FS3000 twin channel analyser, Alpkem, USA). Leachate samples were kept at −20 °C until FIA analyses.

Samples for N_2O flux calculations were taken for both, control and urea treatments. Nitrous oxide concentration was determined using a gas chromatograph (GC) (SRI 8610 gas chromatograph; SRI Instruments, CA, USA) equipped with a ^{63}Ni electron capture detector (ECD) and autosampler (Gilson 222 XL; Gilson Inc., WI, USA). The GC was calibrated using a series of standard gases interspersed with reference standards. The N_2O flux was calculated from the concentration obtained via GC analysis (μL L^{-1}), by integrating the calculated daily N_2O fluxes and linearly interpolating between measurement points for each lysimeter after equation (1) according to (Hutchinson and Mosier, 1981),

$$N_2O\ \text{flux} = \frac{Vc \times P \times (c2 - c0) \times C \times Mw}{Gc(Tk + Tm) \times Ac \times t2} \quad (1)$$

where *Vc* represents the chamber volume of 0.0005 m^3, *P* the atmospheric pressure of 101325 Pa, *c0* and *c2* the N_2O concentration [μL L^{-1}] at time t0 and t2, respectively, *C* the amount of minutes per hour (60), *M* the molecular weight of N_2O-N (28.0134 g mol^{-1}), *Gc* the gas constant (8.314 J K^{-1} mol^{-1}), *Tk* the absolute temperature at 0° (273.15 K), *Tc* the air temperature during measurement (°C), *Ac* the chamber area (0.0057 m^2) and *t2* the total cover period of 40 min. The total N_2O flux (μg N_2O-N m^{-2} h^{-1}) was converted and illustrated as cumulative daily flux per hectare for illustrations and comparison with other N parameters.

A soil nitrification assay performed using the rhizosphere control soil and an $(NH_4)_2SO_4$ solution (10 g of fresh soil in 100 mL working solution). The $(NH_4)_2SO_4$ solution was set up with 1.5 mL of 0.2 M KH_2PO_4 and 15 mL of 50 mM $(NH_4)_2SO_4$, which was diluted to 1 L. The pH of the diluted solution was adjusted to 7.2 by addition of H_2SO_4 and NaOH, respectively. The soil slurry was shaken at 150 rpm and incubated at 28 °C for 4 weeks, with measurements taken for NO_3^- and NH_4^+ after filtering on days 0, 1, 3, 7 and 24.

Statistical analyses were based on n = 4 individual replicates unless stated otherwise. Using SPSS (IBM SPSS statistics 20), data were tested for normality (Kolmogorov-Smirnov) and if necessary, log-transformed prior analyses of variance (ANOVA) followed by Duncan's post-hoc tests to identify homogenous subsets for $\alpha = 0.05$. SigmaPlot 11.0 (Systat Software Inc) was used for illustrations.

3. Results

In both the control and the treatments, the total irrigation for mānuka, kānuka, and pine was 2730, 3140, and 2850 mm respectively (Fig. 1). Leachate was obtained from all species and treatments over the experimental period, although a slightly higher evapotranspiration in combination with lower drainage was observed in the kānuka and mānuka treatments when compared to pine.

Soil pH was not significantly different between plant species within treatments, but was significantly lower after urea application in all treatments (Table 1). At final harvest, soil bulk density and water filled pore space were similar in all plant species in

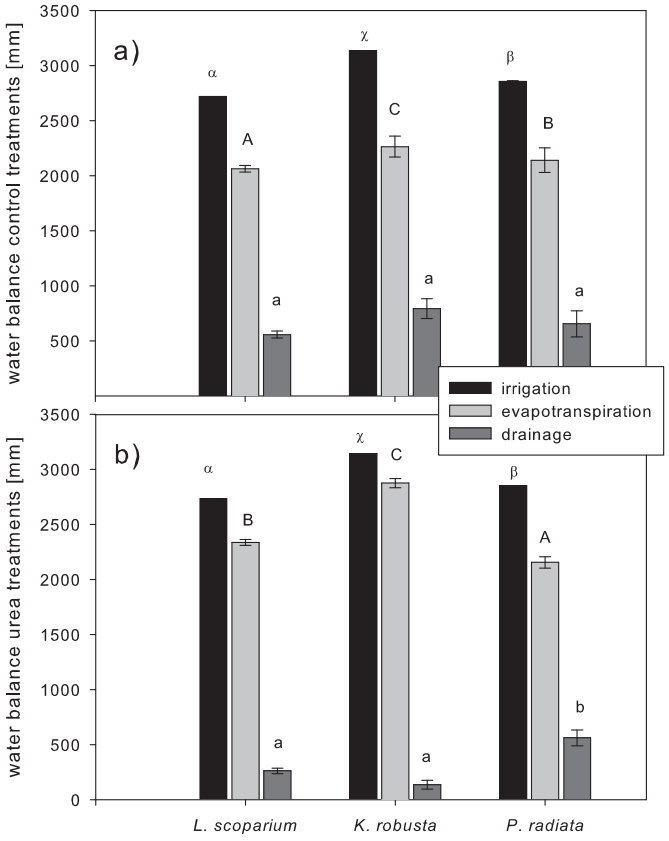

Fig. 1. Irrigation, drainage and evapotranspiration for control (a) and urea (b) treatments [mm], measured over the total experimental period [n = 4 ± se]. Significant differences between plant species at $p \leq 0.05$ are indicated by letters (a, b, c; A, B, C and α, β, γ), considering the same treatment (n = 4 ± se).

control treatments. In urea treatments a lower bulk density was measured in *P. radiata* compared to *L. scoparium*, whereas the water filled pore space was higher in *P. radiata* compared to *L. scoparium*. The water filled pore space and soil moisture content was detected as significantly lower in urea treatments of both, *L. scoparium* and *K. robusta* compared to their corresponding controls. A higher moisture content was measured in *P. radiata* compared to *L. scoparium* or *K. robusta*, irrespective of treatments.

Urea application resulted in significantly increased ($p < 0.05$) aboveground plant biomass (Fig. 2a) and higher N uptake ($p < 0.05$) into plant leaves, stems and roots compared to control treatments, irrespective of plant species (Fig. 2b, c, 2d). Pine, mānuka and kānuka had similar leaf N uptake (Fig. 2b). Lower N content was detected in pine stems (Fig. 2c) compared to both myrtaceae species, whereas higher N content was detected in roots (Fig. 2d).

Cumulative NO_3^--N leached during the 20-day period was <0.5 kg ha^{-1} in plant species controls (Fig. 3a). In both myrtaceae species, ca. 2 kg ha^{-1} NO_3^--N were recovered from urea treatments, whereas pine leached 53 kg ha^{-1} (Fig. 3a). At the end of the experiment, a similar N content (5650–5810 kg ha^{-1}) was measured in the control treatments of all plant species. Urea application increased total N in soil in all plant treatments (6085–6267 kg ha^{-1}). Available N was below the detection limit in control treatments of all plant species (0.1 mg L^{-1}, equivalent to 5 mg kg^{-1} soil). In urea treatments, NO_3^--N was the dominant form of inorganic N in all plant species, although not statistically significant for kānuka (Fig. 3b). Lower amounts of NO_3^--N were detected in urea treatments with kānuka, compared to mānuka and pine. Higher levels of NH_4^+-N were found in both, kānuka and

Table 1
Soil pH, soil bulk density, gravimetric water content and water filled pore space at final harvest [n = 4 ± se]. Significant differences between plant species at $p \leq 0.05$ are indicated by letters (a, b, c), considering the same treatment. Differences between treatments within the same plant species are indicated with asterisks (*).

		control	urea
soil pH	*L.scoparium*	6.28 ± 0.03; $0.03^{a,*}$	5.45 ± 0.06; $0.05^{a,*}$
	K. robusta	6.33 ± 0.02; $0.02^{a,*}$	5.57 ± 0.03; $0.02^{a,*}$
	P. radiata	6.12 ± 0.15; $0.11^{a,*}$	5.38 ± 0.10; $0.08^{a,*}$
soil bulk density [g cm^{-3}]	*L.scoparium*	0.74 ± 0.04^{a}	0.80 ± 0.01^{b}
	K. robusta	0.81 ± 0.04^{a}	0.73 ± 0.02^{ab}
	P. radiata	0.75 ± 0.03^{a}	0.65 ± 0.05^{a}
gravimetric water content [g g^{-1}]	*L.scoparium*	$33.75 \pm 1.18^{a,*}$	$16.75 \pm 2.10^{a,*}$
	K. robusta	$32.50 \pm 1.32^{a,*}$	$23.00 \pm 2.04^{b,*}$
	P. radiata	39.00 ± 0.00^{b}	41.25 ± 1.11^{c}
water filled pore space [cm^3 cm^{-3}]	*L.scoparium*	$0.34 \pm 0.02^{a,*}$	$0.19 \pm 0.02^{a,*}$
	K. robusta	$0.38 \pm 0.03^{a,*}$	$0.23 \pm 0.03^{a,*}$
	P. radiata	0.32 ± 0.11^{a}	0.36 ± 0.04^{b}

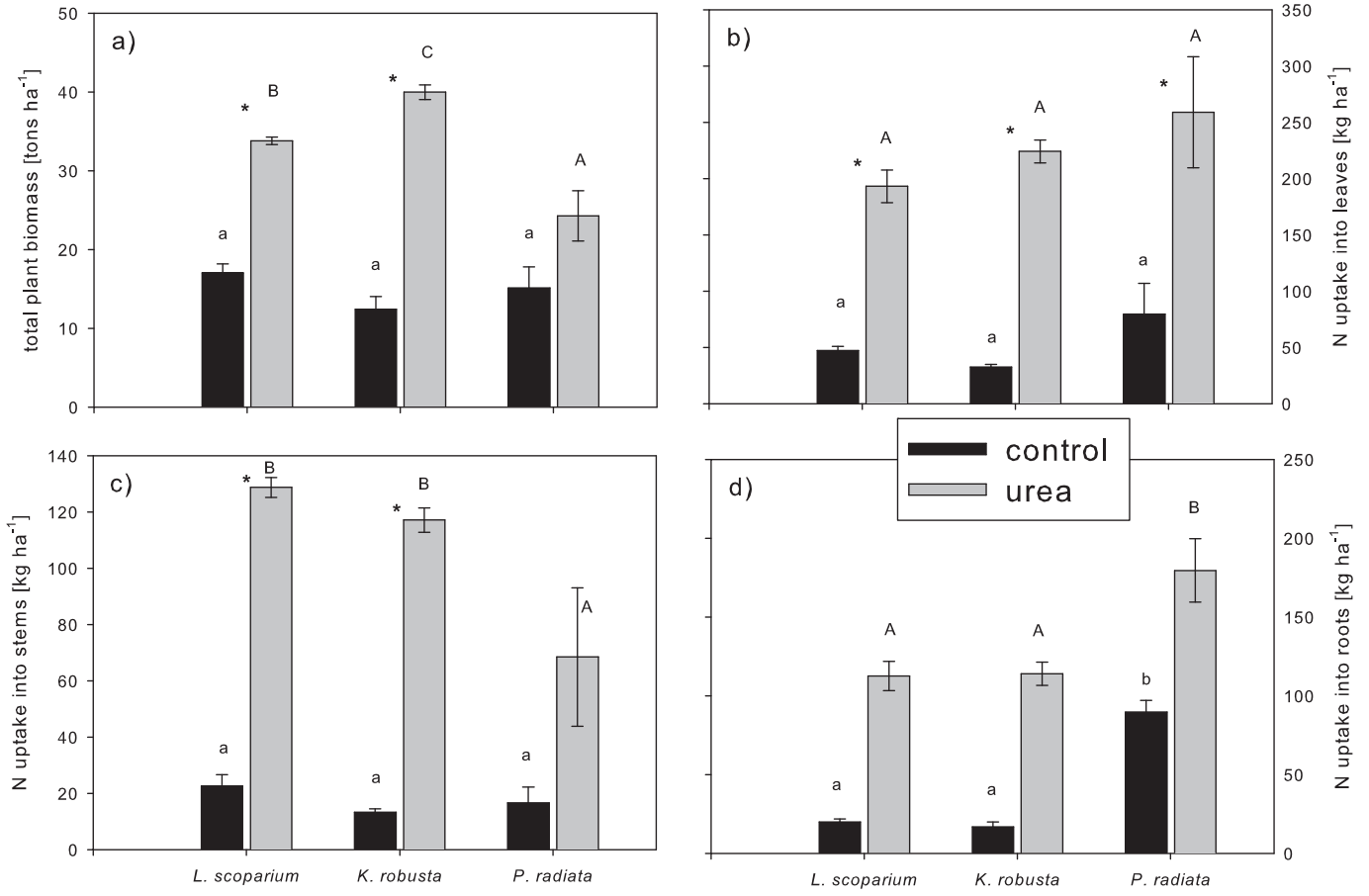

Fig. 2. Plant biomass [tons ha^{-1}] at end of the experiment (a), total N uptake into plant leaves (b), stems (c) and roots (d) in urea compared to control treatments [kg ha^{-1} DW]. Significant differences between treatments at $p \leq 0.05$ are indicated by asterisks (*), whereas letters (a, b, c and A, B, C) show differences between plant species (n = 4 ± se), considering the same treatment.

mānuka compared to pine trees (Fig. 3b).

Results from the nitrification assay showed significantly higher concentrations of NH_4^+-N in kānuka after only one day of assay incubation, and all subsequent incubation measurements (Fig. 4). After an incubation period of 7 days and 14 days, respectively, higher amounts of NH_4^+-N were detected in *K. robusta* compared to *L. scoparium*, whereas samples from *P. radiata* being lowest ($p < 0.05$).

A control N_2O-N flux of <10 μg m^{-2} h^{-1} was measured in combination with all plant species, resulting in a cumulative N_2O-N loss <0.02 kg ha^{-1} (Fig. S2 – supplemental material). Urea application increased N_2O emissions. A moderate increase was measured for all plant species until day 5, where N_2O emissions increased up to 110 μg m^{-2} h^{-1}, 131 μg m^{-2} h^{-1} and 174 μg m^{-2} h^{-1}, in mānuka, kānuka and pine, respectively (Fig. 5a). In pine treatments, continuously high N_2O fluxes were measured until day 17 (468 μg m^{-2} h^{-1}), followed by decreasing fluxes at the end of the measuring period. In kānuka, N_2O flux was mainly in a range between 120 and 170 μg m^{-2} h^{-1}, until day 18, and dropped back to 22 μg m^{-2} h^{-1} at day 20. The N_2O flux measured in urea treatments in combination with mānuka was lowest of all plant species, with a first peak at day 5 (110 μg m^{-2} h^{-1}) and a second peak at day 14 of

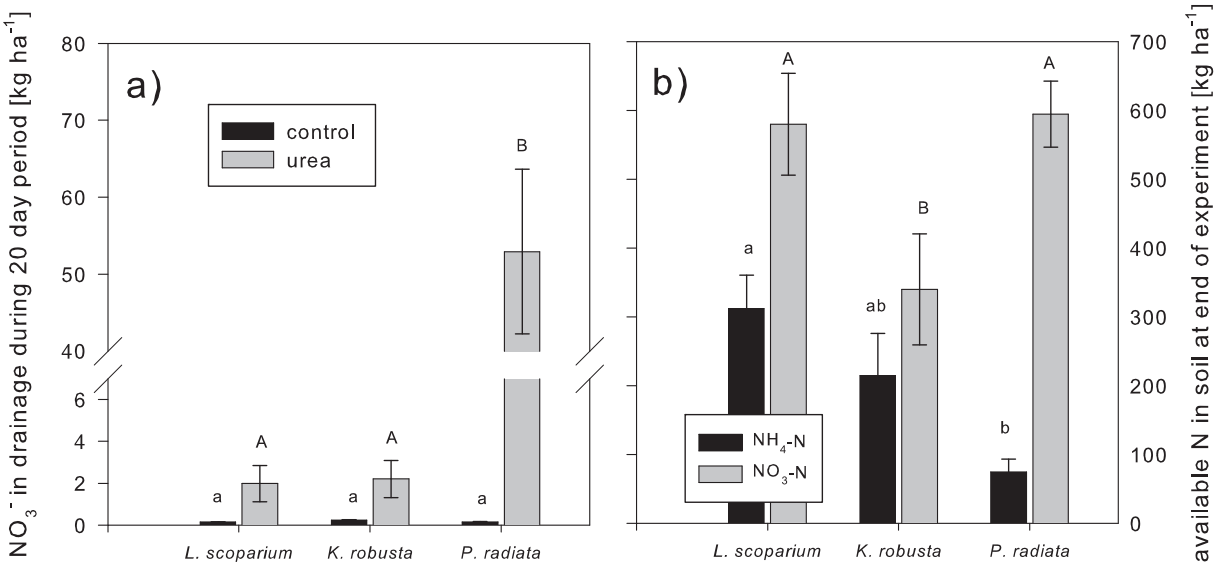

Fig. 3. Total N in soil at the end of the experiment [kg ha^{-1}] in control and urea treatments (a), with NH_4-N and NO_3^- N in soil from urea treatments (b). Significant differences between plant species at $p \leq 0.05$ are indicated by letters (a, b, c and A, B, C), considering the same treatment (n = 4 ± se). Asterisks (*) show differences between treatments.

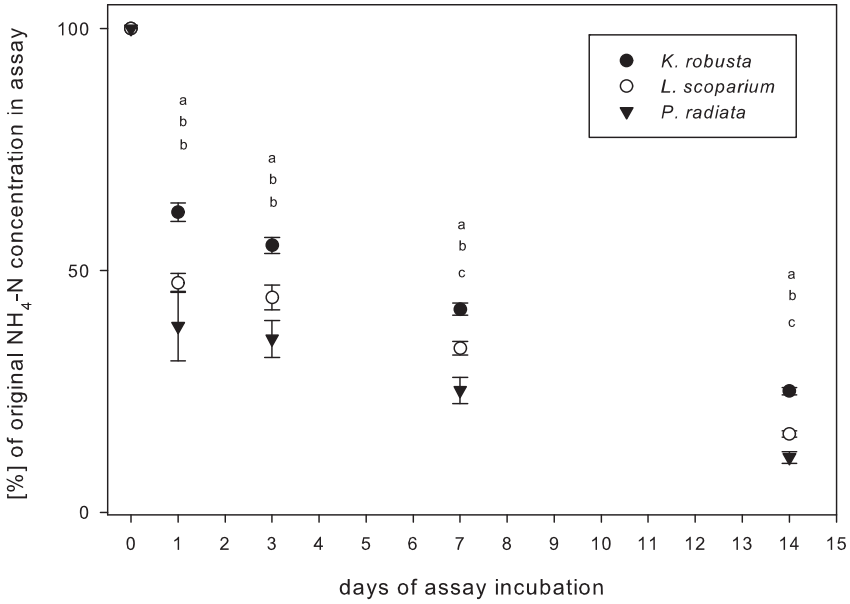

Fig. 4. Decrease of NH_4-N [%] in a soil nitrification assay with control soil over a 14-day incubation period (n = 4 ± se). Initial NH_4-N concentrations were measured with 567.4 (±15.2), 420.1 (±17.6) and 325.1 (±34.8) mg kg^{-1} for *K. robusta*, *L. scoparium* and *P. radiata*, respectively.

the measuring period (120 μg m^{-2} h^{-1}). The cumulative N loss via N_2O over the 20-day period after urea application was 0.82 ± 0.23 kg ha^{-1}, 0.47 ± 0.18 kg ha^{-1} and 0.29 ± 0.05 kg ha^{-1}, in pine, kānuka and mānuka, respectively (Fig. 5b).

4. Discussion

The trees in the control soils all grew without any visible deficiency symptoms. This demonstrates that they are adapted to low fertility soil and marginal land, and hence have potential use in silvopastoral systems, shelterbelts or marginal farm areas (Hawke and Tombleson, 1993). Mānuka and kānuka in such systems show potential as nurse crops for underlying vegetation (Herbert et al., 1997). Urea fertilization increased the aboveground biomass of all tree species, especially kānuka and mānuka. This was accompanied by an increased evapotranspiration and lower drainage, when compared to pine by similar irrigation pattern (2730 mm–3140 mm). The better response of mānuka and kānuka to urea fertilization compared to pine may indicate that these species could potentially remove more excess N from soil and hence reduce N leaching (Mohan Kumar et al., 1998).

After application of 800 kg ha^{-1} of urea, similar N was measured in plant leaves, irrespective of plant species. An increased foliar N content was shown to be beneficial for essential oil production in *Zingiber officinale* (Singh et al., 2016). With increased N fertilization, Tsamaidi et al. (2012) reported an increase in the essential oil yield of dill, containing α-pinene and myrcene, which are key components in mānuka, kānuka and pine essential oils (McDonald et al.,

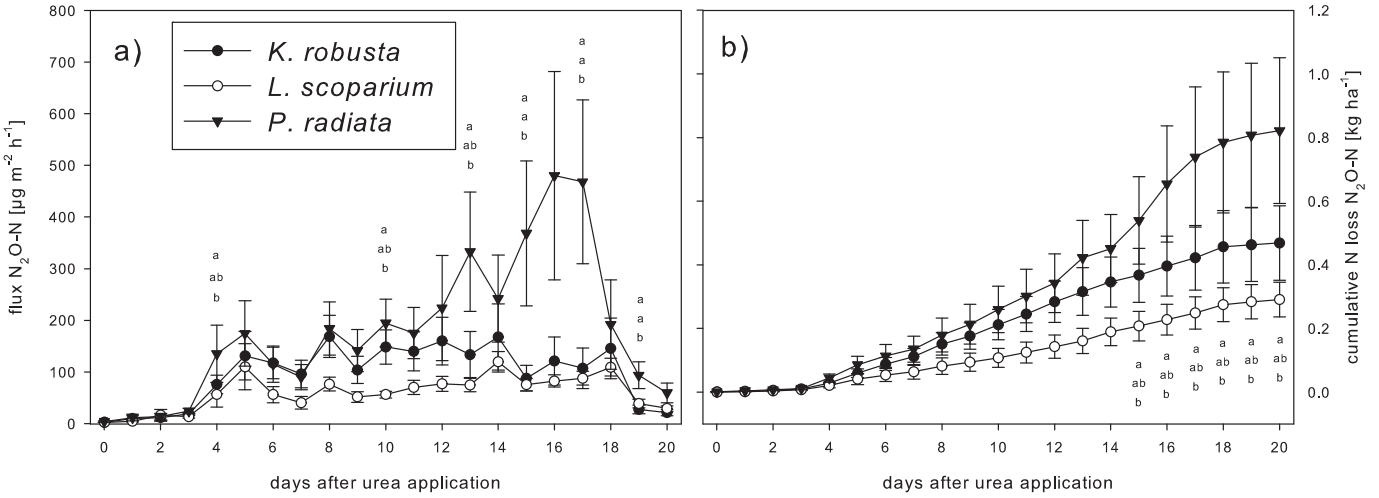

Fig. 5. Urea N_2O flux [µg m^{-2} h^{-1}] and cumulative N_2O loss [kg ha^{-1}] during the 20-day period following urea application (n = 4 ± se). Significant differences $p \leq 0.05$ are indicated by different letters (a, b).

1999; Porter et al., 1998; Porter and Wilkins, 1999). These tree species included in silvopastoral systems could generate revenue via increased amounts and/or quality of essential oils, obtained through N fertilization or via interruption of stock urine patches. Further studies are needed to determine whether the urea changes the quality of any plant products. In N fertilized treatments both mānuka and kānuka allocated 100 kg N ha^{-1} in the stem biomass, compared to only 35 kg ha^{-1} in pine, whereas N allocation belowground was similar for all tree species (90–100 kg ha^{-1}). These results indicate a better response of mānuka and kānuka to urea fertilization regarding harvestable aboveground (timber) products.

At the end of the experiment, a significantly higher total N content was measured in soil after urea application, irrespective of plant species. Similar pH was measured in urea treatments irrespective of plant species; therefore the effect of pH on N loss via NH_3 was negligible. At final harvest, pine treatments showed significantly higher moisture contents compared to kānuka and mānuka, with similar results seen in water filled pore space calculations (Table 1). Analyses of available N showed NO_3^- as the predominant form, especially in pine and mānuka treatments, which suggests a high nitrification potential of the soil used in our experiment. The preference of one form of available N over the other can be quite pronounced for a plant species(Britto and Kronzucker, 2013). Britto and Kronzucker (2013) reported that pine seedlings had greater growth and N uptake with NH_4^+, than NO_3^-, regardless of pH, temperature or type of growth medium. If pine is less efficient at taking up NO_3^-, this could explain the higher amount of NO_3^- in pine treatments in our experiment, compared to only 75 kg ha^{-1} of NH_4^+. A rapid conversion of the applied NH_4-N into NO_3-N hence reduced the preferred N source, and resulted into only low growth response of pine to urea fertilization. Taking into account the low growth response with higher drainage rates in radiata pine, this suggests a potentially higher N loss from pine treatments via leaching, which is confirmed by 53 kg ha^{-1} NO_3-N recovered in pine drainage compared to only 2 kg ha^{-1} in mānuka and kānuka (Fig. S2 – supplemental material).

Kānuka treatments showed high growth response after urea fertilization with significantly lower NO_3-N compared to mānuka and pine, and no significant differences between the two forms NH_4-N and NO_3-N. This could indicate a non-preferential use of available N sources for plant growth. However, higher amounts of NH_4-N in the nitrification assay may also suggest the preference of NH_4-N while at the same time inhibiting nitrification. Since nitrification is a biologically controlled process involving enzymes, mainly ammonia monooxygenase and hydroxylamine oxidoreductase (Robertson and Groffman, 2015), plant species with enzyme inhibiting potential can hence inhibit nitrification (Subbarao et al., 2009). In this context, both, mānuka and kānuka may inhibit nitrification (Kellam et al., 1992), since results from the nitrification assay showed a slower rate of ammonium reduction in the mānuka and kānuka treatments compared to pine, resulting in significantly higher NH_4-N after 7 and 14 days of assay incubation, respectively. These effects are likely to increase competitiveness of kānuka and mānuka over other plant species, and therefore explain and further underline their important role as secondary successional plant species (Franklin et al., 2015; Ross et al., 2009).

High rates of nitrification lead to high emissions of N_2O, especially after rainfall or irrigation (Butterbach-Bahl et al., 2013). This is in accordance with results from our experiments, where a higher N_2O flux and higher cumulative N_2O emission was detected after urea application in combination with pine, compared to mānuka and kānuka. A first peak in N_2O emissions between four and six days indicates N_2O production during the nitrification process, the conversion from NH_4^+ to NO_3^- via NO_2^-, where NO_2^- is reduced to N_2O (Robertson and Groffman, 2015). Although no differences were detected between tree species at this point, a higher cumulative N_2O loss in pine treatments compared to kānuka and mānuka is in accordance with results from our nitrification assay, indicating higher nitrification and N_2O production during nitrification in soil planted with pine trees. A peak of high N_2O emissions was measured 15 days after urea application, most likely because of enzymatic N_2O production during denitrification. In contrast, no further increase or N_2O peak was detected in kānuka and mānuka treatments. This suggests an enzyme inhibiting effect of both, kānuka and mānuka during denitrification. Denitrification is driven by individual enzymes, whereas nitrate reductase inhibits the reduction of NO_3^- to NO_2^-, the first step in the denitrification pathway (Robertson and Groffman, 2015). In the present study, the N content was determined in several plant parts, soil, leachate and gaseous emissions in form of N_2O. Rhizosphere soil was analysed in order to investigate direct effects of different plant species in the same soil type. However, since nitrate and ammonia concentrations in soil vary between rhizosphere and bulk soil (Colin-Belgrand et al., 2003; Zeller et al., 2007), results from this study were not able to provide a complete N mass balance.

5. Conclusions

Both mānuka and kānuka responded positively to the addition of urea. Compared to pine, these species had a significant greater positive response. Reduced N leaching and N_2O emissions from mānuka and kānuka compared to pine indicated that these species may have a similar or better efficacy as pine in reducing nitrification and hence denitrification. These effects could be beneficial in silvopastoral systems, to minimize the amount of N loss via leaching or N_2O emissions, by simultaneously increasing plant and stem biomass and potentially increasing and enhancing aboveground plant products. Future research has to verify the benefits of N fertilization on the quality of plant products, and the effect of plant chemicals on microbial communities and enzymes involved in the N cycle. Field studies including these species in combination with different forage crops will verify results *in situ* and promote alternatives for sustainable silvopastoral systems.

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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.envpol.2017.03.042.

References

Amaral, J.A., Ekins, A., Richards, S.R., Knowles, R., 1998. Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. Appl. Environ. Microbiol. 64, 520–525.
Beitlich, N., Koelling-Speer, I., Oelschlaegel, S., Speer, K., 2014. Differentiation of manuka honey from kanuka honey and from jelly bush honey using HS-SPME-GC/MS and UHPLC-PDA-MS/MS. J. Agric. Food Chem. 62, 6435–6444.
Bergin, D.O., Kimberley, M.O., Marden, M., 1995. Protective value of regenerating tea tree stands on erosion-prone hill country, East Coast, North Island, New Zealand. N. Z. J. For. Sci. 25, 3–19.
Blakemore, L.C., Searle, P.L., Daly, B.K., 1987. Methods for chemical analysis of soils. N. Z. Soil Bur. Sci. Rep. 80, 9–12.
Britto, D.T., Kronzucker, H.J., 2013. Ecological significance and complexity of N-source preference in plants. Ann. Bot. 112, 957–963.
Butterbach-Bahl, K., Baggs, E.M., Dannenmann, M., Kiese, R., Zechmeister-Boltenstern, S., 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? Philosophical Trans. R. Soc. B 368, 20130122.
Castaldi, S., Carfora, A., Fiorentino, A., Natale, A., Messere, A., Miglietta, F., otrufo, M.F., 2009. Inhibition of net nitrification activity in a Mediterranean woodland: possible role of chemicals produced by Arbutus unedo. Plant Soil 315, 273–283.
Chang, S.X., Amatya, G., Beare, M.H., Mead, D.J., 2002. Soil properties under a *Pinus radiata* – ryegrass silvopastoral system in New Zealand. Part I. Soil N and moisture availability, soil C, and tree growth. Agrofor. Syst. 54, 137–147.
Colin-Belgrand, M., Dambrine, E., Bienaimé, S., Nys, C., Turpault, M.-P., 2003. Influence of tree roots on nitrogen mineralization. Scand. J. For. Res. 18, 260–268.
Cooper, A.B., 1986. Suppression of nitrate formation within an exotic conifer plantation. Plant Soil 93, 383–394.
Cuttle, S.P., Bourne, P.C., 1993. Uptake and leaching of nitrogen from artificial urine applied to grassland on different dates during the growing season. Plant Soil 150, 77–86.
Eichhorn, M.P., Paris, P., Herzog, F., Incoll, L.D., Liagre, F., Mantzanas, K., Mayus, M., Moreno, G., Papanastasis, V.P., Pilbeam, D.J., Pisanelli, A., Dupraz, C., 2006. Silvoarable systems in europe – past, present and future prospects. Agrofor. Syst. 67, 29–50.
Franklin, H.M., Dickinson, N.M., Esnault, C.J.D., Robinson, B.H., 2015. Native plants and nitrogen in agricultural landscapes of New Zealand. Plant Soil 394, 407–420.
Hawke, M.F., Tombleson, J.D., 1993. Production and interaction of pastures and shelterbelts in the central North Island. In: Proceedings Oftbe NewZealand Grassland Association, pp. 193–197.
Herbert, J.W., Bergin, D.O., Steward, G.A., 1997. Native Trees for Production Forestry. What's New in Forest Research. New Zealand Forest Research Institute Limited.
Hutchinson, G.L., Mosier, A.R., 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. Soil Sci. Soc. Am. J. 45, 311–316.
Kellam, S.J., Tisch, M.H., Walker, J.R.L., 1992. Screening of New Zealand native plants for enzyme inhibitory activities. N. Z. J. Bot. 30, 199–203.
López-Díaz, M.L., Rigueiro-Rodríguez, A., Mosquera-Losada, M.R., 2009. Influence of pasture botanical composition and fertilization treatments on tree growth. For. Ecol. Manag. 257, 1363–1372.
Maddocks-Jennings, W., Wilkinson, J.M., Shillington, D., Cavanagh, H., 2005. A fresh look at manuka and kanuka essential oils from New Zealand. Int. J. Aromather. 15, 141–146.
McDonald, G.A., Steward, D., Franich, A.R., 1999. Monoterpene composition of radiata pine (Pinus radiata D.Don) sapwood from a 13 year old progeny trial. Holz als Roh- Werkst. 57, 301–302.
Mohan Kumar, B., Jacob George, S., Jamaludheen, V., Suresh, T.K., 1998. Comparison of biomass production, tree allometry and nutrient use efficiency of multipurpose trees grown in woodlot and silvopastoral experiments in Kerala, India. For. Ecol. Manag. 112, 145–163.
Mosquera-Losada, M.R., Fernández-Núñez, E., Rigueiro-Rodríguez, A., 2006. Pasture, tree and soil evolution in silvopastoral systems of Atlantic Europe. For. Ecol. Manag. 232, 135–145.
Mosquera-Losada, M.R., Riguerio, A., McAdam, J., 2005. Silvopastoralism and Sustainable Land Management. CAB International, Wallingford, GB.
Neilsen, W.A., Pataczek, W., Lynch, T., Pyrke, R., 1992. Growth response of Pinus radiata to multiple applications of nitrogen fertilizer and evaluation of the quantity of added nitrogen remaining in the forest system. Plant Soil 144, 207–217.
Northup, R.R., Yu, Z., Dahlgren, R.A., Vogt, K.A., 1995. Polyphenol control of nitrogen release from pine litter. Nature 377, 227–229.
Oenema, O., Velthof, G.L., Yamulki, S., Jarvis, S.C., 1997. Nitrous oxide emissions from grazed grassland. Soil Use Manag. 13, 288–295.
Paavolainen, L., Kitunen, V., Smolander, A., 1998. Inhibition of nitrification in forest soil by monoterpenes. Plant Soil 205, 147–154.
Porter, N.G., Smale, P.E., Nelson, M.A., Hay, A.J., Van Klink, J.W., Dean, C.M., 1998. Variability in essential oil chemistry and plant morphology within a *Leptospermum scoparium* population. N. Z. J. Bot. 36, 125–133.
Porter, N.G., Wilkins, A.L., 1999. Chemical, physical and antimicrobial properties of essential oils of *Leptospermum scoparium* and *Kunzea ericoides*. Phytochemistry 50, 407–415.
Poudel, D.D., Horwath, W.R., Lanini, W.T., Temple, S.R., van Bruggen, A.H.C., 2002. Comparison of soil N availability and leaching potential, crop yields and weeds in organic, low-input and conventional farming systems in northern California. Agriculture, Ecosyst. Environ. 90, 125–137.
Prosser, J., 2011. Manuka (*Leptospermum scoparium*) as a Remediation Species for Biosolids Amended Land. MSc Thesis. Massey University, New Zealand.
Prosser, J.A., Woods, R.R., Horswell, J., Robinson, B.H., 2016. The potential in-situ antimicrobial ability of Myrtaceae plant species on pathogens in soil. Soil Biol. Biochem. 96, 1–3.
Pyke, N., Chynoweth, R., Mathers, D., 2016. Integrated nutrient and water management for sustainable farming. In: Currie, L.D., Singh, R. (Eds.), The Potential Impact and Opportunities from Nutrient Management Regulation on the New Zealand Herbage Seed Industry. Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.
Rice, E.L., Pancholy, S.K., 1973. Inhibition of nitrification by climax ecosystems. II. Additional evidence and possible role of tannins. Am. J. Bot. 60, 691–702.
Robertson, G.P., Groffman, P.M., 2015. Nitrogen transformations. In: Paul, E.A. (Ed.), Soil Microbiology, Ecology and Biochemistry. Academic Press, Burlington, Massachusetts, USA, pp. 421–446.
Ross, D.J., Scott, N.A., Lambie, S.M., Trotter, C.M., Rodda, N.J., Townsend, J.A., 2009. Nitrogen and carbon cycling in a New Zealand pumice soil under a manuka (*Leptospermum scoparium*) and kanuka (*Kunzea ericoides*) shrubland. Aust. J. Soil Res. 47, 725–736.
Selbie, D.R., Lanigan, G.J., Laughlin, R.J., Di, H.J., Moir, J.L., Cameron, K.C., Clough, T.J., Watson, C.J., Grant, J., Somers, C., Richards, K.G., 2015. Confirmation of co-denitrification in grazed grassland. Sci. Rep. 5, 1–9.
Singh, M., Khan, M.M.A., Naeem, M., 2016. Effect of nitrogen on growth, nutrient assimilation, essential oil content, yield and quality attributes in Zingiber officinale Rosc. J. Saudi Soc. Agric. Sci. 15, 171–178.
Song, C.Y., Nam, E.H., Park, S.H., Hwang, C.Y., 2013. In vitro efficacy of the essential oil from Leptospermum scoparium (manuka) on antimicrobial susceptibility and biofilm formation in Staphylococcus pseudintermedius isolates from dogs. Veterinary dermatol. 24, 404–408 e487.
Steinhorn, G., Sims, I.M., Carnachan, S.M., Carr, A.J., Schlothauer, R., 2011. Isolation and characterisation of arabinogalactan-proteins from New Zealand kanuka

honey. Food Chem. 128, 949–956.
Stephens, J.M., Schlothauer, R.C., Morris, B.D., Yang, D., Fearnley, L., Greenwood, D.R., Loomes, K.M., 2010. Phenolic compounds and methylglyoxal in some New Zealand manuka and kanuka honeys. Food Chem. 120, 78–86.
Stephens, J.M.C., Molan, P.C., Clarkson, B.D., 2005. A review of Leptospermum scoparium (Myrtaceae) in New Zealand. N. Z. J. Bot. 43, 431–449.
Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M., Yoshida, M., Rondon, M., Rao, I.M., Lascano, C.E., Berry, W.L., Ito, O., 2009. Evidence for biological nitrification inhibition in Brachiaria pastures. Proc. Natl. Acad. Sci. U. S. A. 106, 17302–17307.
Subbarao, G.V., Sahrawat, K.L., Nakahara, K., Rao, I.M., Ishitani, M., Hash, C.T., Kishii, M., Bonnett, D.G., Berry, W.L., Lata, J.C., 2013. A paradigm shift towards low-nitrifying production systems: the role of biological nitrification inhibition (BNI). Ann. Bot. 112 (2), 297–316. http://dx.doi.org/10.1093/aob/mcs230. https://www.ncbi.nlm.nih.gov/pubmed/23118123.
Suescun, F., Paulino, L., Zagal, E., Ovalle, C., Muñoz, C., 2012. Plant extracts from the Mediterranean zone of Chile potentially affect soil microbial activity related to N transformations: a laboratory experiment. Acta Agriculturae Scandinavica. Sect. B — Soil & Plant Sci. 62, 556–564.
Tsamaidi, D., Karapanos, I.C., Passam, H.C., Daferera, D., Polissiou, M., 2012. The Yield and Composition of Dill Essential Oil in Relation to N Application, Season of Cultivation and Stage of Harvest. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 189–194.
White, C.S., 1988. Nitrification inhibition by monoterpenoids: theoretical mode of action based on molecular structures. Ecology 69, 1631–1633.
Wyatt, R.M., Hodges, L.D., Kalafatis, N., Wright, P.F., Wynne, P.M., Macrides, T.A., 2005. Phytochemical analysis and biological screening of leaf and twig extracts from Kunzea ericoides. Phytotherapy Res. PTR 19, 963–970.
Zeller, B., Recous, S., Kunze, M., Moukoumi, J., Colin-Belgrand, M., Bienaimé, S., Ranger, J., Dambrine, E., 2007. Influence of tree species on gross and net N transformations in forest soils. Ann. For. Sci. 64, 151–158.