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# Influence of plant growth and root architecture of Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*) on N recovery during winter

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## Abstract

Nitrate ($NO_3^-$) leaching is an environmental and health concern. In grazed pasture systems, $NO_3^-$ leaching primarily occurs beneath animal urine patch areas due to high nitrogen (N) loading and the inability of pasture plants to capture all of this N. This study investigated the relative importance of plant growth and root architecture to recover soil N. Herbage N recovery, dry matter (DM) yield and root architecture, following injections of $^{15}$N-enriched urea at different soil depths (5, 25 and 45 cm), were measured for Italian ryegrass (*Lolium multiflorum* Lam.) and tall fescue (*Festuca arundinacea* Schreb.) grown in soil monolith lysimeters (18 cm diameter × 70 cm depth) under simulated South Island, New Zealand winter temperature and light levels. Total herbage N uptake and DM yield were on average 24 and 48% greater in *L. multiflorum* than *F. arundinacea* respectively. Root length density (cm cm$^{-3}$ soil) in the 5- to 25-cm-depth horizon was similar between species. In the 25- to 45-cm-depth horizon, *F. arundinacea* roots were found at higher densities than *L. multiflorum*. In the 45- to 65-cm-depth horizon, root length density was fourfold to ninefold higher for *F. arundinacea* than *L. multiflorum*, but N uptake efficiency was greater in *L. multiflorum* (0·48 mg $^{15}$N m$^{-1}$ root) than *F. arundinacea* (0·09 mg $^{15}$N m$^{-1}$ root). The results suggest that deep *F. arundinacea* roots are relatively inactive during the winter period and confirm that plant growth is more important than root architecture (e.g. deep roots) to recover soil N and ultimately reduce nitrate leaching losses.

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## Introduction

Mitigation techniques are needed to reduce nitrate ($NO_3^-$) leaching losses from pastoral agriculture to prevent environmental and health risks associated with elevated amounts of N in groundwater. The World Health Organisation (WHO, 2007) states that $NO_3^-$-N concentrations in excess of 11·3 ppm in drinking water may lead to blue-baby syndrome in infants. Further, algal blooms are a possible consequence of elevated amounts of N in water bodies and these blooms have the potential to deplete aquatic life. A possible mitigation option is the use of pasture species with suitable root architectures and/or seasonal growth patterns to capture soil N, particularly during cooler winter/early spring months when soils are often draining. Perennial ryegrass (*Lolium perenne* L.) cultivars are commonly used in New Zealand grazed pasture systems; however, they generally have a shallow root system with up to 85% of roots found within the top 15 cm of soil (Troughton, 1957; Haynes and Williams, 1993; Bolinder *et al.*, 2002).

Recent investigations into the strategic use of different pasture species to take up soil N (Crush *et al.*, 2005; Nichols and Crush, 2007; Pirhofer-Walzl *et al.*, 2010; Popay and Crush, 2010; Moir *et al.*, 2013; Malcolm *et al.*, 2014) suggest that alternative pasture species to *L. perenne* may be adopted as a viable strategic option to reduce $NO_3^-$ leaching losses. Malcolm *et al.* (2014) reported that the winter plant growth of *L. multiflorum* was of greater importance than root-system architecture at reducing $NO_3^-$ leaching losses beneath cow urine patches and showed that losses were 24–54% less than other pasture species such as

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deep rooting *F. arundinacea* and *L. perenne*. Similarly, Pirhofer-Walzl *et al.* (2010) suggested that deep roots have less efficient N uptake in deep soil layers compared to N uptake of other shallow-rooted species higher in the soil profile.

Surplus N beneath cow urine patches, when in $NO_3^-$-N form, is highly vulnerable to leaching to depths beyond the 'critical rooting zone' (particularly during winter when soils are draining). It is unclear whether deeper-rooting pasture species compared with species exhibiting greater winter activity (i.e. plant growth/root metabolic activity) are better suited to capturing this N. To further understand these mechanisms, the ability of *L. multiflorum* and *F. arundinacea* [as investigated by Malcolm *et al.* (2014), and differing in root-system architecture and seasonal growth] to take up N from soil depths below the 'critical rooting zone' was investigated.

It was hypothesized that N recovery in the herbage during winter is primarily affected by plant growth (DM accumulation and root metabolic activity) rather than specific root-system architecture. The objective of this experiment was to gain a detailed understanding of the ability of *L. multiflorum* and *F. arundinacea* to recover soil N from different soil depths, and to determine the relative importance of plant growth and root-system architecture in N recovery during the winter period.

## Materials and methods

### Site description

The soil type was a free-draining Templeton fine sandy loam (Immature Pallic soil, Hewitt, 2010; Udic Ustochrepts, Soil Survey Staff, 1998), located on the Lincoln University Research Dairy Farm (LURDF), 15 km south west of Christchurch, New Zealand (43°38′S, 172°28′E; 17 m above sea level). The profile primarily consisted of fine sandy loam and loamy sand textures. This soil supports areas of intensive mixed farming on the Canterbury Plains and accounts for approximately 75 000 ha of the intermediate terraces of Canterbury lowlands (Cox, 1978). Soil cores ($n$ = 50; 0–7·5 cm depth × 2·5 cm diameter) were taken from the trial site area and analysed for soil fertility status (results are given in Table 1).

**Table 1** Initial soil fertility status of the trial sites before basal fertilizer applications of the lysimeter collection area (sampled October 2009).

| Soil property | Value | Methodology |
|---|---|---|
| pH | 6·0 | Water/soil ratio 2·5:1 |
| Olsen P (mg $L^{-1}$) | 27 | Olsen *et al.* (1954) |
| Sulphate S (mg $kg^{-1}$) | 6 | Watkinson and Kear (1994) |
| Total C (% w $w^{-1}$) | 2·6 | LECO CNS-2000 |
| Exchangeable Ca ($cmol_c$ $kg^{-1}$) | 8 | Rayment and Higginson (1992) |
| Exchangeable Mg ($cmol_c$ $kg^{-1}$) | 12 | Rayment and Higginson (1992) |
| Exchangeable K ($cmol_c$ $kg^{-1}$) | 7 | Rayment and Higginson (1992) |
| Exchangeable Na ($cmol_c$ $kg^{-1}$) | 9 | Rayment and Higginson (1992) |
| Anaerobic mineralizable N (kg N $ha^{-1}$)* | 129 | Keeney and Bremner (1966) |

### Trial preparation

Intact soil monolith lysimeters (18 cm diameter × 70 cm depth) were collected from a pre-established pasture site at the Lincoln University Research Dairy Farm, Canterbury, following well-established protocols and procedures outlined in Cameron *et al.* (1992), and installed into a temperature- and light-controlled growth room at the New Zealand Biotron research facility at Lincoln University, Canterbury. In brief, the lysimeter collection involved placing a PVC cylinder casing (19 cm diameter with 1 cm internal cutting ring × 70 cm depth) on the soil surface, carefully digging around the casing to avoid structural damage to the soil core inside and gradually pushing the casing down by small increments. When the casing reached the desired depth (*ca.* 70 cm), the soil monolith was sliced off at the base. An end cap was then secured to the base of the lysimeter, and petroleum jelly was used to seal the gap between the soil core and casing to prevent preferential edge flow. The lysimeters were then lifted out and transported to the Lincoln University Biotron facility on a specially designed trailer with air-bag suspension to minimize disturbance. The bottom 5 cm of soil was replaced with gravel to create a condition similar to that in the field and to prevent sediment accumulating in the drainage tube. End caps were then fixed and sealed to the base of the lysimeters so that drainage water passed out the hole in the centre of the base cap. Lysimeters were randomly placed inside the Biotron growth chamber on 90 × 90 cm width × 50 cm height wooden tables. Plastic tubing was connected to the base of each lysimeter, which fed into plastic 2 L drainage water collection vessels.

All the lysimeters received basal fertilizer applications at the beginning of the trial which consisted of 50 kg P $ha^{-1}$ in the form of 20% potash sulphur super fertilizer (0-10-6-16). The lysimeters also received the equivalent of 2 t $ha^{-1}$ of hydrated lime.

Four Campbell Scientific Water Content Reflectometer CS615 moisture probes and four 107 Campbell Scientific temperature sensors were inserted into randomly allocated lysimeters and recorded data every 10 min. The data were collected using a CR23X Campbell Scientific data logger and are presented as weekly averages.

## Growth chamber and climate

The experiment was conducted in a Conviron BDW120 plant growth room from December 2011 until May 2012 (simulating May until October: late autumn/winter/early spring). The lighting system consisted of 48 × 400 W metal halide bulbs (Venture Ltd) in combination with 48 × 100 W incandescent bulbs (Phillips® soft tone, soft white) mounted above a Perspex barrier 2·4 m above floor level. For the majority of the experiment, photosynthetically active radiation (PAR) at the top of the plant canopy was 450 μmol $m^{-2}$ $s^{-1}$ (achieved using 30 metal halides in combination with 48 incandescents) to simulate winter light intensities between the hours of 07:30 and 17:30. For the later part of the experiment, full lighting was used (730 μmol $m^{-2}$ $s^{-1}$ at plant canopy height) to simulate spring/summer light intensities and day length progressively increased to 12 h per day. Temperature was controlled and adjusted on a weekly basis to simulate changing conditions between the cool months of May and October and was set to replicate equivalent Canterbury diurnal patterns. A top-down airflow pattern with sufficient outdoor air was maintained to sustain ambient $CO_2$ conditions within the room. Daytime relative humidity was maintained at 70% (±3%), and night time humidity at 80% (±3%).

Average weekly volumetric water content ranged from 32 to 40 g $cm^{-3}$ (Figure 1). From early June until early October (simulated months), weekly soil water content varied by a maximum of 7%. Soil temperature data are also shown in Figure 1. Average weekly soil temperature was lowest in late June at 5·7°C and steadily rose to *ca.* 13·8°C in the final week of September.

## Treatments

The trial was arranged as a completely randomized 2 × 3 factorial design (six treatments) with four replicates of each treatment (24 lysimeters). Two pasture species treatments [1. *Lolium multiflorum* (Italian ryegrass, cv. 'Tabu') and 2. *Festuca arundinacea* (tall fescue, cv. 'Advance')] and three depths of $^{15}N$ injection (5, 25 and 45 cm below the soil surface) were imposed.

The pasture treatments were sown in March 2010 following conventional cultivation (i.e. plough, harrow and roll), 21 months prior to the collection of the lysimeters (early December 2011). The pastures were excluded from livestock grazing and managed as per local best-management practice. At sowing, seeds of both pasture species were sown at the equivalent rate of 25 kg $ha^{-1}$, along with *Trifolium repens* (white clover, cv. 'Kopu II' large leaf) at 3 kg $ha^{-1}$ in the mixture. These pasture species were chosen because of their different attributes (e.g. root-system architecture and seasonal growth pattern) and to better understand the mechanism behind the results obtained in the study of Malcolm *et al.* (2014).

The $^{15}N$ injection treatments were administered as a dissolved $^{15}N$-enriched urea (10 atom %) solution, which was uniformly injected through the sidewalls of the lysimeters at a rate of 300 kg N $ha^{-1}$ on 15 May (simulated date). This was performed using specifically

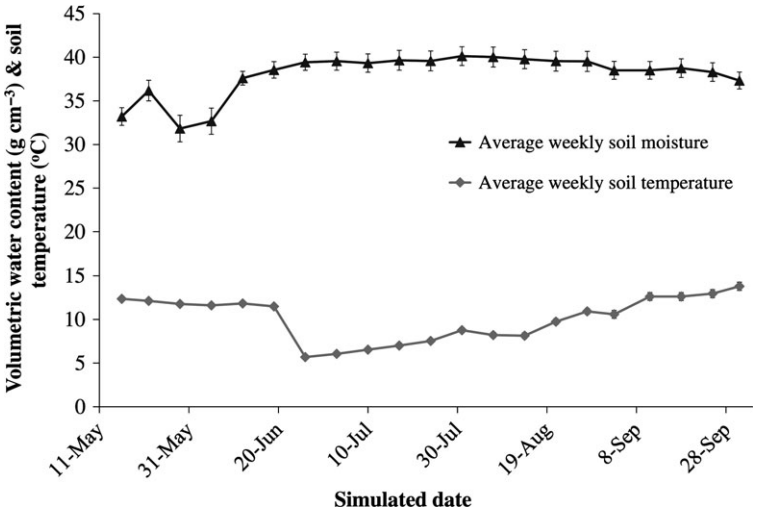


**Figure 1** Average volumetric water content (g $cm^{-3}$) and average weekly soil temperature (°C) following $^{15}N$ injections under simulated conditions from May to October. Vertical bars indicate the s.e.m.

designed needles for each injection point around the lysimeter. There were seven injection points at each injection depth of 5, 25 or 45 cm. The injection points consisted of 1·6-mm-diameter holes running parallel with the centre hole. The centre hole was drilled in line with the centre point of the lysimeter. The urea solution was administered in a grid pattern to achieve an even distribution of $^{15}$N-enriched solution throughout the soil at the given depth. This was performed using a measurement board with a series of marked points, indicating where an injection burst was to be administered. Single injections bursts consisted of 4 mL of the $^{15}$N-enriched solution which was based upon calculations of estimated pore space within the immediate vicinity of the injection point at the tip of the needle. This method was first tested by injecting red dye into a mock lysimeter which was then cut in two approximately one centimeter below the injection point and visually assessed to confirm uniformity of solution penetration.

All lysimeters were irrigated 2–3 times per week with deionized (DI) water. Applications were made to a weekly target rate derived from data collected from the soil moisture probes, estimated evapotranspiration rates, and average weekly Canterbury rainfall data from the NIWA Broadfield EWS (Electronic Weather Station, agent number 17603). The water was applied to the lysimeters with a hand-held irrigation nozzle connected to a submersible pump and a calibrated run timer.

## Measurements

### *Herbage*

Swards within the lysimeters were harvested four times during the trial period, simulating grazing events through the winter/spring period. Cuts were made with electric hand shears, and all lysimeters were harvested to a height of *ca.* 5 cm, i.e. the best-management practice for post-grazing sward height.

Herbage wet and dry weights were obtained for each lysimeter sample, and these measurements were used to calculate herbage DM production. Samples were then dried (70°C), finely ground and then analysed for total N/$^{15}$N content by a stable isotope ratio mass spectrometer (GSL/20-20, Sercon Ltd, Crewe, UK), following combustion at 1000°C in an automated Dumas style elemental analyser. The amount of $^{15}$N recovered was calculated using the formula given by Cabrera and Kissel (1989). The equation used was:

$$^{15}\text{N recovered } (\%) = 100\,p(c - b)/f(a - b), \qquad (1)$$

where $p$ = moles of N in plant material, $f$ = moles of N in the injected urea solution, $c$ = atom % $^{15}$N abundance in the plant, $a$ = atom % $^{15}$N in the urea solution and $b$ = atom % $^{15}$N abundance of plants grown in unfertilized soil. The recovery of the $^{15}$N is then expressed as a percentage of the total amount of $^{15}$N which was injected at the beginning of the experiment.

### *Leachate*

Drainage water was collected from the lysimeters on a fortnightly basis following the N injections for approximately 5 months. This involved measuring the volume of drainage water and obtaining a 100 mL sample for $NO_3^-$-N concentration analysis. Drainage water samples were then analysed by flow injection analysis (FIA) for $NO_3^-$-N concentration (Gal *et al.*, 2004). From the analyses, $NO_3^-$-N leaching from each lysimeter treatment was determined.

$^{15}$Nitrate-N in the leachate was recovered by the diffusion method described by Brooks *et al.* (1989), and samples were then analysed on a stable isotope ratio mass spectrometer (GSL/20-20, Sercon Ltd, Crewe, UK). This enabled the calculation of the percentage of injected $^{15}$N in the leachate as $NO_3^-$-N using the equation given by Cabrera and Kissel (1989), as described above.

### *Root architecture*

At the end of the trial, all lysimeters were destructively sampled for root-architecture analysis. Soil samples of 10 × 10 cm width × 20 cm depth were taken from each core between the depths of 5–25, 25–45 and 45–65 cm. These were then put through a root washer to isolate the root material from the soil. This involved placing individual soil samples into 300-μm cages which rotated on mechanical rollers for approximately 10 min while water jets directed at the cages washed most of the soil away leaving root material inside (Benjamin and Nielsen, 2004). Root samples were further carefully washed by hand to remove any remaining soil residue. Clean root samples were then measured for root length and root surface area by the computer scanner and software package WinRHIZO (Reg V2009c; Regent Instruments Inc., Quebec City, QC, Canada) (Himmelbauer *et al.*, 2004), derived from digitized grey-scale images [400 dpi, with a transmitted light unit (TLU), Epson Expression 10000XL 3.49 (Epsom America, Inc., Long Beach, California, USA)].

Average root length density is reported and defined as root length (cm) per unit volume of soil. Root $^{15}$N uptake efficiency (mg $^{15}$N m$^{-1}$ of root) is defined as the total amount of $^{15}$N recovered in the herbage of all cuts, per unit of root length measured between the N injection depth and the base of the lysimeter. Root length density (cm cm$^{-3}$) was used to calculate total

root length for the soil horizon between each N injection point down to 65 cm depth. The approach used for calculating root $^{15}N$ uptake efficiency enabled comparisons of root activity between pasture species (i.e. root metabolic activity) within the relevant soil horizon, assuming $^{15}N$ was not taken up by plant roots above the point at which N was administered. In other words, roots above the depth of injection were not accounted for when calculating root $^{15}N$ uptake efficiency.

### Statistical analysis

Data sets were statistically analysed by analysis of variance (ANOVA) for a completely randomized trial using GenStat (14th edition, Lawes Agricultural Trust). This tested for treatment effects of the variability in total herbage N uptake, $^{15}N$ recovery in the herbage and drainage water, DM yield, root length density, root surface area and total $NO_3^-$-N leached. Root length density differences of pasture species responses to injection depth were explored independently for data collected at each of three sampled soil horizons. Where necessary, data sets were log-transformed to ensure homogeneity of residuals. An indication of the variability associated with estimated treatment effects was provided by the least significant difference (LSD) with $\alpha = 0{\cdot}05$. In addition, the relationship between injection depth and N leached was assessed using least squares regression, allowing for both linear and polynomial responses.

## Results

### Herbage N uptake, $^{15}N$ recovery and yield

There was a significant ($P < 0{\cdot}01$) main effect of pasture species and a highly significant ($P < 0{\cdot}001$) main effect of injection depth on total N uptake (Figure 2). Total N uptake decreased with increasing soil injection depth, decreasing from 187 to 70 kg N ha$^{-1}$ for *L. multiflorum* and from 161 to 60 kg N ha$^{-1}$ for *F. arundinacea*. On average, *L. multiflorum* took up 24% more N than *F. arundinacea* ($P < 0{\cdot}01$) and was significantly ($P < 0{\cdot}05$) higher for N injections made at the 25 cm depth compared with the same depth treatment for *F. arundinacea*.

There was a significant ($P < 0{\cdot}01$) main effect of pasture species on herbage $^{15}N$ recovery and a highly significant ($P < 0{\cdot}001$) main effect of N injection depth (Table 2). $^{15}$Nitrogen recovery ranged from a low of 9% (*F. arundinacea*; 45 cm injection depth) to a high of 40% (*L. multiflorum*; 25 cm injection depth). *Lolium multiflorum* recovered 43% more $^{15}N$ than *F. arundinacea* in the 25 cm injection depth treatment ($P < 0{\cdot}05$).

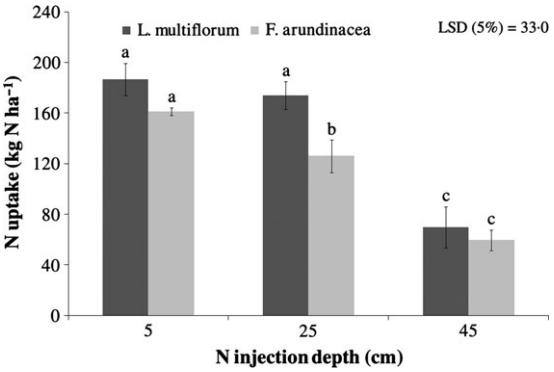


**Figure 2** Total N uptake (kg N ha$^{-1}$) following $^{15}N$ injections at three depths (5, 25 and 45 cm). Vertical bars indicate the s.e.m. Means with a letter in common are not significantly different at the 5% level. LSD, least significant difference.

Pasture species and N injection depth had highly significant ($P < 0{\cdot}001$) main effects on herbage DM production, ranging from 2·6 t DM ha$^{-1}$ (*F. arundinacea*; 45 cm $^{15}N$ injection depth) to 8·0 t DM ha$^{-1}$ (*L. multiflorum*; 5 and 25 cm $^{15}N$ injection depths) (Figure 3). On average, *L. multiflorum* produced 48% more herbage DM than *F. arundinacea* ($P < 0{\cdot}05$) and differences of the species were significant in the 5 and 25 cm N injection depth treatments. When N was injected at the 45 cm depth, herbage DM yields of *L. multiflorum* and *F. arundinacea* were 48–51% less than those of the 5 and 25 cm injection depth treatments ($P < 0{\cdot}05$).

### Drainage and nitrate-N recovery in leachate

Total drainage water collected from the lysimeters ranged from 388 to 542 mm (Table 3). There was a significantly ($P < 0{\cdot}05$) greater volume (*ca.* 14%) of drainage water collected from the *F. arundinacea* treatment (443 mm) compared with *L. multiflorum* where N was injected at the 25 cm depth. There was a highly significant ($P < 0{\cdot}001$) main effect of injection depth on the amount of drainage water, and a highly significant ($P < 0{\cdot}001$) linear and significant ($P < 0{\cdot}01$) quadratic relationship between drainage and N injection depth. The largest volumes of drainage water were collected beneath treatments receiving N at 45 cm depth, which were significantly greater than drainage water volumes collected from the treatments receiving N at 5 and 25 cm depth.

Total $NO_3^-$-N leaching losses ($\log_{10}$) are given in Table 3. There was no significant difference between pasture species; however, there was a highly significant ($P < 0{\cdot}001$) linear and significant ($P < 0{\cdot}01$) quadratic effect of N injection depth on total $NO_3^-$-N

**Table 2** Herbage recovery (%) and total uptake (mg) of $^{15}N$, and uptake efficiency (mg $^{15}N$ $m^{-1}$ root) for the different N injection depths.

| Species treatment | N injection depth (cm) | Herbage $^{15}N$ recovery (%) | Total $^{15}N$ uptake (mg) | Root uptake efficiency (mg $^{15}N$ $m^{-1}$ root) |
|---|---|---|---|---|
| *Lolium multiflorum* | 5 | 38·26 | 32·48 | 0·10 |
| | 25 | 39·87 | 33·85 | 0·34 |
| | 45 | 13·09 | 11·11 | 0·48 |
| *Festuca arundinacea* | 5 | 32·57 | 27·65 | 0·05 |
| | 25 | 27·86 | 23·65 | 0·11 |
| | 45 | 9·06 | 7·70 | 0·09 |
| LSD (5%) | | 8·42 | 7·15 | 0·25 |
| Main effect means | | | | |
| | 5 | 35·40 | 30·07 | 0·08 |
| | 25 | 33·90 | 28·75 | 0·23 |
| | 45 | 11·10 | 9·40 | 0·28 |
| LSD (5%) | | 5·96 | 5·06 | 0·18 |
| Pasture species | | | | |
| *L. multiflorum* | | 30·40 | 25·81 | 0·31 |
| *F. arundinacea* | | 23·20 | 19·67 | 0·08 |
| LSD (5%) | | 4·86 | 4·13 | 0·14 |

LSD, least significant difference.

leaching losses. The average total $NO_3^-$-N leaching loss where N injections were performed at 5 cm depth was *ca.* 1 kg N $ha^{-1}$, and increased to 44 and 90 kg N $ha^{-1}$ at 25 and 45 cm injections depths respectively.

Similarly, there was no significant effect of pasture species on $^{15}N$ recovery in the leachate ($log_{10}$), but a highly significant ($P < 0·001$) linear and significant ($P < 0·01$) quadratic effect of N injection depth (Table 3). On average, 0·1% of injected $^{15}N$ at 5 cm depth was recovered in the leachate. This significantly ($P < 0·05$) increased to 4·1 and 8·6% at N injection depths of 25 and 45 cm respectively.

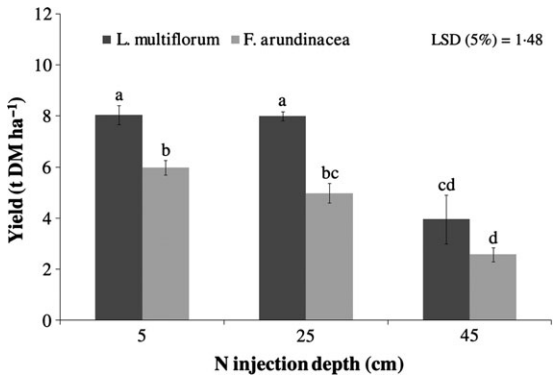


**Figure 3** Total dry matter (DM) production (t DM $ha^{-1}$) following $^{15}N$ injections at three depths (5, 25 and 45 cm). Vertical bars indicate the s.e.m. Means with a letter in common are not significantly different at the 5% level. LSD, least significant difference.

## Root architecture

The root length densities (cm $cm^{-3}$ soil) of *L. multiflorum* and *F. arundinacea* treatments at the 5–25, 25–45 and 45–65 cm depth horizons are shown in Figure 4. Root length density was highest in the 5–25 cm depth horizon, ranging from 4·3 (*L. multiflorum*; Figure 4c) to 6·9 cm $cm^{-3}$ (*F. arundinacea*; Figure 4a). Root length densities declined with increasing depth for both pasture species. For all three injection depth treatments, the root length density of *F. arundinacea* in the 45- to 65-cm-depth horizon was significantly ($P < 0·05$) higher than *L. multiflorum* by fourfold to ninefold. Of the 45 cm N injection depth treatments, the difference in density between pasture species in the 45- to 65-cm-depth horizon was the greatest, where root length density of *F. arundinacea* was sixfold higher than *L. multiflorum*. Root length density of *F. arundinacea* was also significantly ($P < 0·05$) greater than *L. multiflorum* in the 25- to 45-cm-depth horizon where injections were made at 5 cm depth (Figure 4a).

The total surface area of recovered roots from the lysimeters is illustrated in Figure 5. There was a significant ($P < 0·01$) main effect of pasture species on total surface area. Total surface area ranged from 794 $cm^2$

**Table 3** Total nitrate-N leached, $^{15}N$ recovery in leachate and drainage following $^{15}N$ injections at three different depths.

| Treatments | | Total nitrate-N leached (kg $NO_3^-$-N $ha^{-1}$) | | $^{15}N$ recovery in leachate (%) | | |
|---|---|---|---|---|---|---|
| Pasture species | Injection depth (cm) | $Log_{10}$ (mean) | Back-transformed mean | $Log_{10}$ (mean) | Back-transformed mean | Drainage (mm) |
| *Lolium multiflorum* | 5 | −0·015 | 0·97 | −0·981 | 0·10 | 408·0 |
| | 25 | 1·659 | 45·60 | 0·571 | 3·72 | 388·1 |
| | 45 | 1·989 | 97·50 | 0·957 | 9·06 | 542·3 |
| *Festuca arundinacea* | 5 | 0·063 | 1·16 | −0·732 | 0·19 | 424·1 |
| | 25 | 1·617 | 41·40 | 0·647 | 4·44 | 442·8 |
| | 45 | 1·920 | 83·18 | 0·909 | 8·11 | 522·4 |
| LSD (5%) | | 0·565 | – | 0·520 | – | 52·0 |
| LSR (5%) | | – | 3·68 | – | 3·31 | – |
| Main effect means | | | | | | |
| | 5 | 0·024 | 1·06 | −0·856 | 0·14 | 416·0 |
| | 25 | 1·638 | 43·45 | 0·609 | 4·06 | 415·4 |
| | 45 | 1·954 | 89·95 | 0·933 | 8·57 | 532·3 |
| LSD (5%) | | 0·400 | – | 0·368 | – | 36·8 |
| LSR (5%) | | – | 2·51 | – | 2·33 | – |
| Pasture species | | | | | | |
| *L. multiflorum* | | 1·211 | 16·26 | 0·182 | 1·52 | 463·1 |
| *F. arundinacea* | | 1·200 | 15·85 | 0·275 | 1·88 | 446·1 |
| LSD (5%) | | 0·327 | – | 0·300 | – | 30·0 |
| LSR (5%) | | – | 2·12 | – | 2·00 | – |
| Significance of 3*2 factorial contrasts | | | | | | |
| Main effect of injection depth | | | | | | |
| Linear (L) | | *** | | *** | | *** |
| Quadratic (Q) | | ** | | ** | | ** |
| Main effect of pasture species (P) | | n.s. | | n.s. | | n.s. |
| Interaction contrasts | | | | | | |
| L*P | | n.s. | | n.s. | | n.s. |
| Q*P | | n.s. | | n.s. | | n.s. |

n.s., not significant; LSR, least significant ratio; LSD, least significant difference.*$P < 0·05$; **$P < 0·01$; ***$P < 0·001$.

(*L. multiflorum*; 45 cm N injection depth) to 1761 $cm^2$ (*F. arundinacea*; 5 cm N injection depth). Total surface area of *F. arundinacea* roots was up to 110% greater than *L. multiflorum* roots where N solution was injected at 5 and 45 cm depths ($P < 0·05$).

### Root N-uptake efficiency

Pasture species had a significant ($P < 0·01$) main effect on root N-uptake efficiency (Table 2). The highest and lowest efficiencies were measured in the 45 cm N injection depth treatment, where *L. multiflorum* efficiency was 0·48 mg $^{15}N\ m^{-1}$ of root compared to 0·09 mg $^{15}N\ m^{-1}$ under *F. arundinacea*. On average, root N-uptake efficiency of *F. arundinacea* was *ca.* 73% less than *L. multiflorum* ($P < 0·01$).

## Discussion

Nitrogen uptake and $^{15}N$ recovery in the herbage were generally higher from *L. multiflorum* than *F. arundinacea* grown under simulated late autumn/winter/early spring temperature and rainfall conditions. This result agrees with the study of Malcolm *et al.* (2014) conducted outdoors using lysimeters, where $NO_3^-$ leaching losses were on average 42% lower under *L. multiflorum* compared with *F. arundinacea*. These results confirm the potential of *L. multiflorum* to reduce $NO_3^-$ leaching losses from grazed pasture systems.

It is important to note that, in New Zealand, *L. multiflorum* is often used as a short-term species, either sown as a single grass species in a pasture

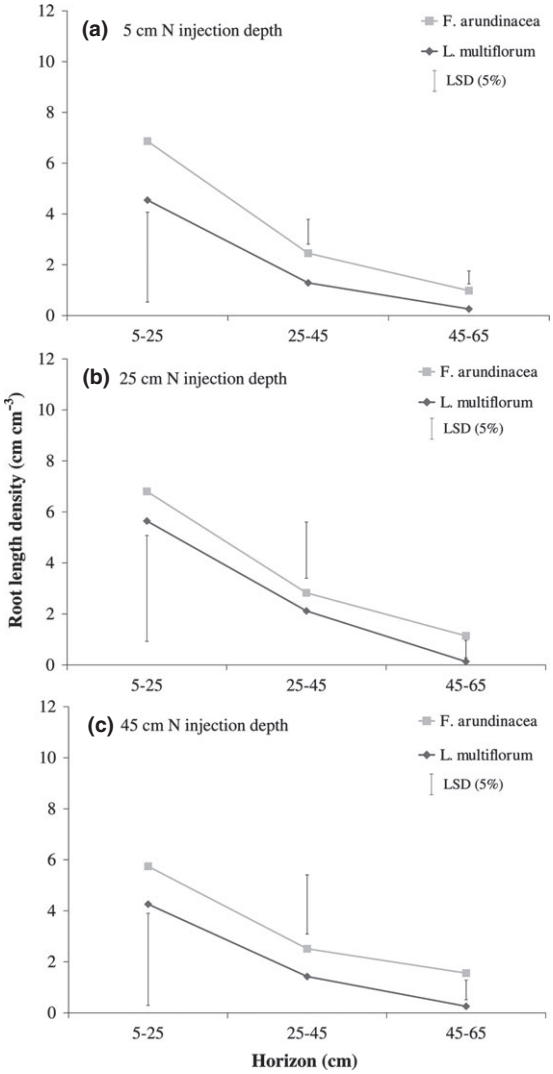


**Figure 4** Root length densities (cm $cm^{-3}$) at three sampled horizons following injections of $^{15}N$ enriched urea solution at (a) 5 cm depth, (b) 25 cm depth and (c) 45 cm depth. Least significant difference (LSD) ($P < 0{\cdot}05$) calculated for two treatment means at each depth separately.

mixture [i.e. with white clover (*Trifolium repens*)] or sown into existing perennial pastures that have low production. However, with adequate irrigation and N fertilizer, and when insect pest pressure is low, *L. multiflorum* has been shown to be productive for 2–3 years before subsiding. In this study where both swards were between 18 and 24 months of age, there were no signs of senescence. This is evident in Figure 3 where DM yields of *L. multiflorum* were in most cases higher than the perennial species *F. arundinacea*.

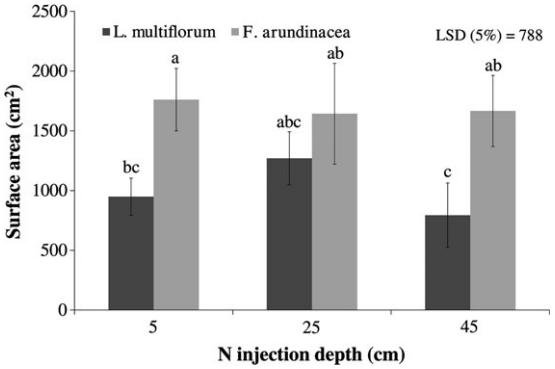


**Figure 5** Total surface area ($cm^2$) of recovered root material at the end of the trial period. Vertical bars indicate the s.e.m. Means with a letter in common are not significantly different at the 5% level. LSD, least significant difference.

There was a distinct effect of N injection depth on N uptake, $^{15}N$ recovery and herbage DM yield, with notably lower levels recorded when N was injected at the 45 cm depth. This was attributed to the larger volume of drainage water collected from the 45 cm N injection depth treatments, leading to higher leaching losses (Table 3) and consequently lower N availability and plant N uptake.

Total N uptake and $^{15}N$ recovery of both pasture species showed similar trends at each N injection depth. Statistically significant differences were seen in the 25 cm injection depth treatments where *L. multiflorum* took up 38% more N and recovered 43% more $^{15}N$ than *F. arundinacea*. A study by Moir *et al.* (2013) indicated that total N uptake by *L. multiflorum* following surface N applications of 300 kg N $ha^{-1}$ was 62–90% higher than *F. arundinacea* (cv. 'Advance' and 'Flecha'). Similarly, Crush *et al.* (2005) showed that the proportion of surface-applied $^{15}N$ solution taken up by *L. multiflorum* was approximately double that of *F. arundinacea*. Although these two studies were carried out under warmer glasshouse conditions, they support the fact that *L. multiflorum* has an added ability to take up more available soil N, even at depths below 25 cm.

Detailed root analyses of *L. multiflorum* and *F. arundinacea* by Malcolm *et al.* (2014) indicated that a greater root length density may not necessarily increase N uptake during winter. Our study confirms these findings and shows that there are considerable differences in N-uptake efficiency of roots between the two studied pasture species, particularly with increasing depth. There was no significant difference in the proportion of $^{15}N$ recovery between *L. multiflorum* and *F. arundinacea* pasture species where N injections were made at the 45 cm depth, despite *F. arundinacea* con-

taining fivefold higher root length densities than *L. multiflorum* in the 45- to 65-cm-depth horizon. In this horizon, *L. multiflorum* had a root uptake efficiency of 0·48 mg $^{15}N\ m^{-1}$ of root compared with 0·09 mg $^{15}N\ m^{-1}$ by *F. arundinacea*. Although differences were not regarded as being statistically significant for the other injection depth treatments, root uptake efficiency of *L. multiflorum* was notably higher than *F. arundinacea*. This suggests that during the winter period when plants are less active, root metabolic activity of *L. multiflorum* is high compared with *F. arundinacea*, and *L. multiflorum* consequently has as much ability to exploit N reserves from deep in the soil as *F. arundinacea*. These results are in agreement with Crush *et al.* (2005) who showed that *L. multiflorum* roots were more efficient than *F. arundinacea* roots at N uptake, and reported $^{15}N$ uptake (mg $g^{-1}$ root weight) of *L. multiflorum* to be 10% higher than *F. arundinacea* following surface applications of $^{15}N$ solution in a warmer climate.

*Lolium multiflorum* produced more herbage DM than *F. arundinacea* following N injections at 5 and 25 cm depths, which has most likely contributed to the higher N uptake and $^{15}N$ recovery by *L. multiflorum*. This supports the findings of Malcolm *et al.* (2014) where they reported up to 119% greater winter DM yield from *L. multiflorum* pasture than *F. arundinacea*. Similarly, other studies have also shown that *L. multiflorum* can produce considerably higher yields than other perennial species (Ridley and Simpson, 1994; Crush *et al.*, 2005; Popay and Crush, 2010; Moir *et al.*, 2013).

Nitrate leaching losses following N injections close to the soil surface were notably lower than those in various other studies (Di and Cameron, 2005, 2007; Menneer *et al.*, 2008; Malcolm *et al.*, 2014). This was attributed to the lower rate of administered N [e.g. 300 kg N $ha^{-1}$ cf. 1000 kg N $ha^{-1}$ used by Malcolm *et al.* (2014)]. Moir *et al.* (2013) also reported $NO_3^-$-N leaching losses beneath *L. multiflorum* to be similar to those found in this trial following surface applications of 300 kg N $ha^{-1}$, but were notably higher beneath *F. arundinacea* at 82 kg $NO_3^-$-N $ha^{-1}$. This was likely because of significantly lower DM yields of *F. arundinacea* treatments, as well as greater rates of mineralization and nitrification as the experiment was carried out under warmer glasshouse conditions.

Our work also supports a recent study by Pirhofer-Walzl *et al.* (2010), investigating the N uptake of shallow- vs. deep-rooted plants in a multispecies and monoculture grassland using a $^{15}N$ tracer placed at three soil depths (40, 80 and 120 cm). They concluded that deep-rooted species (e.g. *Medicago sativa* and *Cichorium intybus*) are not more efficient in N uptake in deep soil layers, and do not add a 'deepness' function to communities with shallow roots. Although the depths in that study were generally greater than those in ours, the principles may still apply given the depths of interest are below the 'critical rooting zone' (Malcolm *et al.*, 2014), where N is highly susceptible to leaching.

White clover was used as part of the seed mixture due to its common use in New Zealand grazed pasture systems; however, there was very little clover present in the harvested material above ground (<1%), probably due to the low temperatures. Various authors have hypothesized that legumes present in the pasture sward can supply additional atmospheric N to sward grasses, therefore reducing the requirement for deep-rooted grass species to explore N resources in deeper soil layers (Rasmussen *et al.*, 2007; von Felten *et al.*, 2009). However, the activity of white clover during the winter period (particularly within urine patch areas where N is often at excessive levels) is likely to be minimal and therefore may only supply a relatively small amount of N to the plant. Although measured clover levels were low, it is likely that clover was present in the pasture swards below the height of harvest as stolons and roots, given the monoliths were collected during the early summer period when clover is actively growing. In which case, it is inevitable that some of the roots recovered were in fact clover roots; however, it is unlikely that differences in observed root length densities were attributed to the presence of clover.

High urinary-N input [e.g. Malcolm *et al.* (2014)] may reduce the potential benefit of deep roots, particularly in the few weeks following urine deposition when N remains within the 'critical rooting zone.' That aside, our study has confirmed that higher winter growth (herbage DM accumulation and root metabolic activity) is more critical than specific root architecture (e.g. deep roots) as a key driver to capture soil N from various depths in the soil profile [which ultimately reduces $NO_3^-$ leaching losses as indicated in the study of Malcolm *et al.* (2014)]. It should be recognized that a significant amount of further research into other pasture species with high winter growth is required.

## Conclusions

The results show that the higher plant growth of *L. multiflorum* was responsible for *ca.* 24% more N taken up by the sward, on average over all injection depth treatments, than *F. arundinacea*. In addition, when N was injected at 25 cm depth, *L. multiflorum* recovered 43% more $^{15}N$ than *F. arundinacea*. Similarly, *L. multiflorum* treatments produced on average 48% more herbage DM than the respective *F. arundinacea* treatments, and differences between the species were significant in the 5- and 25-cm N injection depth

treatments. Where N was injected at the 45 cm depth, root length density of *F. arundinacea* was sixfold higher than *L. multiflorum* in the 45- to 65-cm-depth horizon. Of the 45 cm N injection depth treatments, *L. multiflorum* had a root uptake efficiency of 0·48 mg $^{15}N\ m^{-1}$ of root compared with 0·09 mg $^{15}N\ m^{-1}$ by *F. arundinacea*, suggesting that *F. arundinacea* roots were relatively inactive during winter at this depth. We therefore accept the hypothesis that N recovery in the herbage during winter is most strongly affected by plant growth (DM accumulation and root metabolic activity) rather than specific root architecture. We suggest that it is critical to select and focus future research on plant growth as a way to capture soil N during the winter period, to ultimately reduce soil $NO_3^-$ leaching losses.

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