Cadmium Uptake by Ryegrass and Ryegrass–Clover Mixtures under Different Liming Rates

Ebrahim Benyas, Jennifer Owens, Salome Seyedalikhani, and Brett Robinson*

Abstract
Cadmium accumulates in soils that receive repeated applications of Cd-rich superphosphate fertilizers. There is evidence that adding clovers to a crop solubilizes soil Cd, increasing the bioavailability of Cd. This can lead to high plant Cd concentrations. This research aimed to test whether liming-induced increases in pH in mixed crops of clovers and ryegrasses reduced forage Cd concentrations. A greenhouse pot trial applied lime at three rates (0, 1, and 2% of soil dry weight) to eight different plant treatments—four as monocultures (perennial ryegrass [Lolium perenne L.], Italian ryegrass [L. multiflorum Lam.], white clover [Trifolium repens L.], and red clover [T. pratense L.]) and four as ryegrass–clover mixtures (two plant types in each treatment)—in soil (initial soil pH = 5.1, initial soil Cd concentration = 1.31 mg kg⁻¹) with added Cd (CdSO₄) to 1 mg kg⁻¹. Adding lime increased soil pH in both mono- and mixed crops and, in most treatments, increased forage yields. However, the relationship between forage Cd and soil pH differed between plant treatments. In mono- and mixed crop treatments containing perennial ryegrass, adding lime increased the forage yield but did not increase the mass of Cd in the plants compared with the no-lime treatment. However, adding lime to treatments that included Italian ryegrass increased both the forage yield and the Cd compared with the no-lime treatment. The results show that a combination of certain plant species composition and lime rates can optimize forage yields without increasing forage Cd concentrations.

Core Ideas
• Liming increased plant yield in all plant treatments.
• Liming decreased plant Cd uptake in mixed crops that included perennial ryegrass.
• Liming increased plant Cd uptake in mixed crops that included Italian ryegrass.
• Managing Cd uptake requires the correct pairing of lime with both soil and pasture.

Cadmium is a contaminant of phosphate fertilizer (Kabata-Pendias and Mukherjee, 2007). Repeated application of superphosphate fertilizer can result in Cd accumulation in agricultural soils (Loganathan et al., 2003). Accumulation of soil Cd can be slowed by monitoring and controlling fertilizer inputs (Mortvedt, 1995; Roberts and Longhurst, 2002); however, there is no practical method of removing Cd from soil (Robinson et al., 2009). Soil Cd levels of 1 mg kg⁻¹ are regarded as polluted (Fay, 2007). Once in the soil, Cd can be taken up by plants and enter the food chain when contaminated plants are grazed by livestock (Lee et al., 1996; Cadmium Working Group, 2011). Over time, livestock grazing high-Cd plants will accumulate Cd in their liver and kidneys, and consumption of Cd-contaminated offal meats can have detrimental effects on human health and compromise exports of offal products (Cadmium Working Group, 2011). For example, offal meat from pasture-grazed New Zealand sheep older than 30 mo is not fit for human consumption due to the risk of high Cd levels (Cadmium Working Group, 2011). Strategies to minimize the ingestion of Cd by grazing animals include selecting forage species that have low Cd uptake and creating soil conditions that minimize the plant availability of Cd (Simmler et al., 2013).

Rates of Cd uptake by plants depend on soil Cd concentrations, soil organic matter content, soil clay content, soil moisture conditions, availability of macro- and micronutrients, soil pH, and plant species (Grant et al., 1999; Welch and Norvell, 1999). Others have found that increased soil pH of soil amended with Cd salts (CdSO₄) decrease plant Cd concentrations in corn (Zea mays L.; Street et al., 1978; Mahler et al., 1987; López-Chuken and Young, 2010) and Swiss chard (Beta vulgaris L.; Mahler et al., 1987), but that there were species-specific differences in Cd uptake. Cadmium salts may result in greater Cd uptake by plants that would be observed in nonamended Cd-contaminated soils (López-Chuken and Young, 2010). Nitrogen-fixing legumes such as clovers can acidify the soil around the rhizosphere, which may solubilize soil Cd (Liu et al., 2012). This infers that the presence of a leguminous species in pastures may increase Cd uptake by neighboring nonleguminous plants (Li et al., 2009, 2013).
Liu et al., 2013). However, rhizosphere acidification in such mixed-crop systems may also improve nutrient availability and plant growth in alkaline soils (Latati et al., 2014). Pasture species composition varies, but many temperate grazed pastures are cultivated with perennial ryegrass (Pr, *Lolium perenne* L.) and white clover (Wc, *Trifolium repens* L.), and some include red clover (Re, *T. pratense* L.) may be increasingly used in grazed pastures due to its potential to reduce NO, leaching (Malcolm et al., 2015). A mixed crop of legumes like clovers and nonlegumes like ryegrass as forage for grazing livestock may improve plant yields and nutrition and may also result in higher plant Cd concentrations.

A potential strategy to reduce plant Cd uptake in mixed legume and nonlegume systems may be to reduce soil Cd solubility by increasing soil pH with lime additions (Bolan et al., 2003a). Differences in Cd uptake by individual plant species and their individual effects on the soil environment will influence the environmental conditions and therefore Cd solubilization and uptake. How liming mixed legume (clover) and nonlegume (ryegrass) systems affects plant Cd concentrations in the context of grazed pasture management must be tested. Results from past studies indicate that the rates of plant Cd uptake vary with plant species (An et al., 2011; Liu et al., 2012). Therefore, the objectives of this study were to test whether:

1. The presence of clovers in clover–ryegrass mixed-crop systems increases forage Cd. We hypothesized that N fixation by the clover species would acidify soil substrates thereby decreasing soil pH and increasing forage Cd.
2. Liming soil negates the effects of the presence of clovers, thereby reducing forage Cd in clover–ryegrass mixed-crop systems.
3. The treatments of mixed ryegrass–clover crops and lime additions increase forage yields.

**Materials and Methods**

**Experimental Design**

A pot trial was conducted in a greenhouse at Lincoln University, New Zealand, with six replicates for each treatment in a complete randomized block design. The soil had one of three levels of lime added (0, 1, and 2% of lime for the total dry mass of soil), and one of eight plant treatments. The plant treatments were monocrops of perennial ryegrass (Pr), Italian ryegrass (Ir), white clover (Wc), or red clover (Re), or ryegrass–clover mixed crops of Pr+Wc, Pr+Re, Ir+Wc, or Ir+Re.

For all monocrop treatments, 40 seeds were planted per pot, and 16 plants were kept for the duration of the experiment. For the mixed-crop treatments, 40 ryegrass and 20 clover seeds were planted, and 16 ryegrass plants and eight clover plants were kept; the density ratio of ryegrass to clovers was 2:1. The plants were left to grow for 45 d in the greenhouse. The plants were not treated with inoculum at planting since the soil was harvested from a field that had a long history of clover establishment.

**Soil Collection and Preparation**

Soil was collected from an established pasture at the Lincoln University Commercial Dairy Farm (43°38′11.35″ S, 172°26′17.00″ E). The soil is classified as typic immature pallic soil according to the New Zealand Soil Classification (Hewitt, 2010; Landcare Research, 2018). The chemical properties of the soil prior to treatment applications are listed in Table 1. Plant material and visible roots were removed from the soil, and the soil was sieved to <20 mm. Both Cd and lime were added at the start of the experiment (Day 1) when the soil was added into the pots.

**Cadmium Addition to Soil**

For Cd additions, 456 mg CdSO₄ was dissolved in 2 L deionized water and divided into three portions of 0.66 L. Each portion of this Cd solution was separately poured on 83.33 kg wet soil at 25% soil moisture (66.66 kg dry soil equivalent). The CdSO₄ solution was added to soil to ensure that it contained sufficient Cd concentrations for the experiment (Table 2).

**Lime Additions**

Lime (solid limestone CaCO₃, Thermo Fisher Scientific New Zealand) was added as a percentage of the total dry mass of soil to achieve liming concentrations of 1 and 2%. No lime was added to the 0% lime treatment.

For the 1 and 2% lime treatments, 0.667 and 1.334 kg of lime were added to 83.33 kg wet soil (66.66 kg dry soil equivalent), respectively. Assuming a bulk density of 1.0 g cm⁻³ and 50-cm soil depth, the 1 and 2% liming rates were equivalent to field rates of ~40,000 and ~80,000 kg ha⁻¹.

In the soil treatments that received lime (1 and 2%), the soil had already received the CdSO₄ solution, and for each lime treatment, the lime and soil were homogenized in a concrete mixer.

**Pot Preparation**

Pots were filled with treated soil to a volume of 1.25 L, ~640 g dry weight equivalent of soil per pot. Each pot was irrigated with tap water every other day for the first 2 wk of the experiment.

**Table 1. The physicochemical properties of the soil prior to treatment applications showing the elements, and their concentrations with units in parentheses.**

<table>
<thead>
<tr>
<th>Properties</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clay/silt/sand (%)†</td>
<td>4/20/76</td>
</tr>
<tr>
<td>pH (H₂O)†</td>
<td>5.1</td>
</tr>
<tr>
<td>Cation exchange capacity (cmol, kg⁻¹)</td>
<td>12.3</td>
</tr>
<tr>
<td>Soil C (%)†</td>
<td>3.3</td>
</tr>
<tr>
<td>Soil N (%)†</td>
<td>0.3</td>
</tr>
<tr>
<td>Cd (mg kg⁻¹)</td>
<td>1.31</td>
</tr>
<tr>
<td>Al (mg kg⁻¹)</td>
<td>24,720</td>
</tr>
<tr>
<td>Cu (mg kg⁻¹)</td>
<td>4.65</td>
</tr>
<tr>
<td>Fe (g kg⁻¹)</td>
<td>12.4</td>
</tr>
<tr>
<td>K (g kg⁻¹)</td>
<td>4.75</td>
</tr>
<tr>
<td>Mg (g kg⁻¹)</td>
<td>3.86</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>369</td>
</tr>
<tr>
<td>Na (mg kg⁻¹)</td>
<td>338</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>718</td>
</tr>
<tr>
<td>Pb (mg kg⁻¹)</td>
<td>12.6</td>
</tr>
<tr>
<td>S (mg kg⁻¹)</td>
<td>336</td>
</tr>
<tr>
<td>Zn (mg kg⁻¹)</td>
<td>61.7</td>
</tr>
<tr>
<td>Ca (g kg⁻¹)</td>
<td>3.44</td>
</tr>
</tbody>
</table>

† These properties were taken from previous research using the same soil as was used in this research (Simmler et al., 2013)
During irrigation, water was manually added to each pot until the soil water content of each pot reached approximately field capacity. Each pot had four holes in the bottom to prevent pooling of water after irrigation. The soil pH and extractable metals for each soil treatment prior to plants being added are presented in Table 2. Due to a limited research budget, only one plant treatment was assessed for soluble chemicals after the plants were harvested, and Pr was chosen due to its prevalence as a grazed pasture species.

Chemical Analysis of Soil and Plants

At the end of the growing period, the plants were harvested, washed with tap water and then distilled water, and oven dried at 70°C for 72 h to acquire plant dry mass to represent forage yield. Immediately after harvesting the plants, the pots were sacrificed, and the soil was mixed and then subsampled for measurements. The plants were not separated into above- and belowground portions because our primary concern was the measurement of Cd from soil by plants rather than where the Cd in the plant was stored. Soil pH was measured from a subsample of soil that was air dried for 3 d, and the rest of the soil was oven dried at 50°C for 1 wk.

The soil pH was determined from a mixture of soil and deionized water at a ratio of 1:2.5. The mixture was stirred and left to settle for 24 h (Blakemore et al., 1987) and then measured using a pH meter (SevenEasy, Mettler Toledo). The terms “rhizosphere soil” and “rhizosphere soil pH” refer to the soil in the pot after plants were established.

Cation exchange capacity of the soil was measured using the 0.01 M silver thiourea (AgTU) method (Blakemore et al., 1987). This included mixing on an end-over-end shaker 0.70 g of dry soil with 35 mL of 0.01 M AgTU in a 50-mL vial for 16 h, followed by centrifuging for 10 min at 2000 rpm. The supernatant was filtered through a Whatman No. 40 filter, and analysis was completed using a Varian 720 ES inductively coupled plasma-optical emission spectrometer (ICP-OES).

The total C and N were used to report the percentage C, percentage N, and C/N ratio. They were measured from soil samples using an Elementar Vario-Max CN elemental analyzer.

The extractable concentrations of elements, including Cd, of soil and plant material were determined using acid digests analyzed on the ICP–OES. Dried soil (0.5 g) was digested in 5 mL HNO₃ and 1 mL 30% H₂O₂ (Merck Group). Samples were left overnight in a fume cupboard and then digested using a microwave digestion at 175°C for 20 min. After cooling, the samples were diluted with deionized water to a volume of 25 mL and filtered with a Whatman No. 52 filter paper. For plant material, 0.2 g of dry biomass was acid digested and extracted in 5 mL of HNO₃ and 1 mL of H₂O₂ and was subsequently diluted to a volume of 10 mL with deionized water. Reference soil and plant material (International Soil analytical Exchange [ISE] 921 and International Plant analytical Exchange [IPE] 100) from Wageningen University, the Netherlands, were analyzed for quality assurance. Recoverable concentrations were 91 to 108% of the certified values.

Extractable element concentrations for the soil treatments were determined from Ca(NO₃)₂ extracts on the ICP–OES. Dried soil (0.5 g) was weighted into a 50-mL tube, and 30 mL of 0.05 M Ca(NO₃)₂ was added (Gray et al., 1999a; Black et al., 2012). Tubes were shaken on the end-over-end shaker for 2 h, then centrifuged at 10000 rpm for 10 min and filtered with a Whatman No. 52 filter paper.

For the Pr monocrop treatment, the extractable elements in the soil were measured both before the plants were added to the soil and after the plants were harvested at the end of the experiment. The extractions from the soil represent the solute that was extractable from the soil using the simple salt extracts employed in this study.

Data Analysis

Forage Cd is represented in concentrations (mg Cd kg⁻¹) and mass of Cd in the forage for each pot (mg Cd pot⁻¹) to compare how the potential mass of Cd that may be consumed by cattle.
which varied by forage yield. A two-way ANOVA (plants species × lime concentration) was used to test for between group differences of dry mass forage yields and concentrations of Cd in dry plants (mg kg⁻¹), and a Tukey’s post hoc test at 0.05 probability levels was performed. This analysis was completed using SPSS 16 (SPSS, 2007).

Differences in extractable soil elements before planting and after harvesting from the Pr monocrop treatment were calculated. This was done to detect whether there was an increase or decrease in extractable elements associated with the presence of Pr plants.

Pearson’s correlations were completed to assess the strength of the relationships between forage Cd concentrations per unit dry weight of forage (ng Cd kg⁻¹), and plant variables and soil pH using R Statistics version 3.3.0 (R Core Team, 2013). For this analysis, data were pooled by plant treatments, and correlations were run for each plant treatments using data from all three liming rates (0, 1, and 2% lime). Significance was established at P ≤ 0.05.

**Results**

**Effects of Clovers on Soil and Forage Cadmium**

Adding plants to lime-free soil increased soil pH by ~0.5 units compared with the soil before planting. The soil pH in the lime-free mixed crops was not significantly different from that in lime-free monocrop ryegrasses treatments (Fig. 1A). This is contrary to the original hypothesis that the addition of clover to ryegrasses would decrease soil pH.

Plants had a significant effect on forage Cd concentrations (P < 0.001). In the 0% lime treatment, forage Cd in Wc was significantly lower than in Rc (Fig. 1B). Within the 0% lime treatments, there was no significant difference in forage Cd concentrations between Ir monocrops and Ir mixed crops (Ir+Rc and Ir+Wc), and there were no significant differences between Pr monocrops and Pr mixed crops (Pr+Rc and Pr+Wc) (Fig. 1B).

The masses of forage Cd within each pot in the 0% lime treatments were similar in Pr (0.00061 mg pot⁻¹) and Ir (0.00067 mg pot⁻¹), and forage Cd concentrations were generally lower in the mixed-crop treatments that included Wc (Pr+Wc and Ir+Wc) compared with the treatments that included Rc (Pr+Rc and Ir+Rc) (Fig. 1C).

**Effects of Lime Addition on Forage Cadmium Concentrations**

The addition of 1% lime increased soil pH by 1.7 to 1.8 units compared with the soil before planting (Fig. 1A, Table 3). Soil pH in the 2% lime treatment was between 7.5 and 7.7 in all treatments (Fig. 1A). Soil pH in the 2% lime treatment for the mixed crops was 0.1 units lower than for the monocrop ryegrasses (Ir and Pr). Lime treatments had a significant effect on forage Cd concentrations (P < 0.001). A significant interaction between the plant treatments and lime treatments on forage Cd concentrations was detected (P < 0.001). Forage Cd concentration was significantly higher in the Ir 2% lime treatment than in the Ir 0% lime treatment (Table 3, Fig. 1B). The monocrop Rc, Wc, and Pr forage Cd showed small decreases with the addition of lime, but greater limiting rates (2 vs. 1%) did not consistently result in lower forage Cd in these treatments (Table 3, Fig. 1B).

Adding lime to the mixed crops tended to decrease forage Cd concentrations compared with the 0% lime treatment. However, adding more lime (2 vs. 1%) did not further reduce forage Cd concentrations in Pr+Rc or Ir+Wc (Fig. 1B). The Ir+Wc treatment was the only treatment where greater lime additions increased forage Cd concentrations. Despite the increases in forage yield (Fig. 1D), the Pr mono- and mixed crop masses of forage Cd were similar at all liming rates, ranging from 0.000498 to 0.000768 mg Cd pot⁻¹ (Fig. 1C). In the Ir mono- and mixed-crops, both forage yields and plant Cd concentrations increased, and masses of forage Cd ranged from 0.00026 to 0.00193 mg Cd pot⁻¹.

**Effects of Liming on Soil Element Solubility**

Adding lime to soil, and adding lime to soil, likely influenced the solubility of elements in the soil. In Pr, Cd and most other measured extractable elements decreased with increasing lime rates before Pr was added to the soil and after Pr was harvested, including Cd (Table 2). Despite increased lime application decreasing the solubility of many of the elements, many of the extractable elements were lower in the soil after Pr was harvested compared with before the plants were harvested. These elements included Cd, Al, Fe, Mn, Na, and S (Table 2).

**Effects of Liming and Plant Species on Forage Yields**

Both plant treatments (P < 0.001) and lime treatments (P < 0.001) had an effect of forage yield, and there was a significant interaction between plant treatment and lime treatment on forage yield (P = 0.029). Within the 0% lime treatment, there was no statistical difference in forage yields between any of the plant treatments (Fig. 1D). When comparing yield from within the 1 and 2% lime treatments individually, there were differences by plant treatments.

Adding lime at rates of 1 and 2% generally increased the forage yield in all plant treatments compared with 0% lime; however, not all increases were statistically significant. Reductions in mean forage yields occurred in the Ir at the 1% liming rate, where there was a 1% decrease in forage yield compared with the 0% lime treatment, and the Wc monocrop, where forage yields were lower with 2% lime than with 1% lime (Table 3, Fig. 1C). Lime application had a greater effect on forage yields in the mixed-crop treatments that included Ir compared with those with Pr (Fig. 1C). The greatest increase in forage yield from the mixed-crop treatments was observed in the Ir+Rc treatment at 2% lime, which showed a 44% increase in yield compared with the 0% treatment (Table 3).

**Relationships among Forage Yield, Cadmium Uptake, and Other Nutrients**

In the mono-crop clover treatments (Wc and Rc), there were negative relationships between forage yield and forage Cd concentrations (Table 4). The greatest reduction in forage Cd concentrations and increase in forage yields with lime application was observed in the Rc mono-crop where adding liming increased forage yields by more than 30% and decreased forage Cd concentrations by more than 50%, compared to the 0% lime treatment (Table 3). While there was no significant relationship observed between those variables in the Pr mono-crops, there was a positive relationship between forage yield and forage Cd concentrations in the Ir mono-crop. In the mono-crop treatments, there were some similarities in the relationships between
the forage Cd concentrations and other plant elements (Table 4).
In the mixed-crop treatments, there were weak positive relationships between forage yield and forage Cd concentrations, except in the Pr+Rc treatment, where the relationship between the two variables was negative (Table 4).

**Discussion**

Compared to the ryegrass mono-crops, mixing clover and ryegrasses had little influence on soil pH (Fig. 1A), and did not always lead to significantly higher forage Cd concentrations.

**Table 3. Average (± SE) difference in soil pH and percentage difference of dry mass yield of plants and forage Cd between 0 and 1% and 0 and 2% lime applications for perennial ryegrass (Pr), Italian ryegrass (Ir), white clover (Wc), red clover (Rc), and mixed-crop treatments. Negative values indicate decreases.**

<table>
<thead>
<tr>
<th>Plant treatment</th>
<th>Difference between lime treatments</th>
<th>Soil pH</th>
<th>Forage weight</th>
<th>Cd dry forage concentration</th>
<th>Cd dry forage concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 vs. 1%</td>
<td>0 vs. 2%</td>
<td>0 vs. 1%</td>
<td>0 vs. 2%</td>
<td>0 vs. 1%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr</td>
<td>1.7 (0.02)</td>
<td>2.0 (0.03)</td>
<td>8 (11)</td>
<td>22 (14)</td>
<td>−15 (12)</td>
</tr>
<tr>
<td>Ir</td>
<td>1.6 (0.01)</td>
<td>2.0 (0.02)</td>
<td>5 (21)</td>
<td>21 (17)</td>
<td>22 (23)</td>
</tr>
<tr>
<td>Wc</td>
<td>1.8 (0.03)</td>
<td>2.2 (0.02)</td>
<td>30 (9)</td>
<td>12 (8)</td>
<td>−51 (18)</td>
</tr>
<tr>
<td>Rc</td>
<td>1.7 (0.02)</td>
<td>2.0 (0.02)</td>
<td>33 (4)</td>
<td>37 (6)</td>
<td>−52 (7)</td>
</tr>
<tr>
<td>Pr+Wc</td>
<td>1.8 (0.02)</td>
<td>2.0 (0.02)</td>
<td>29 (6)</td>
<td>38 (6)</td>
<td>−10 (5)</td>
</tr>
<tr>
<td>Pr+Rc</td>
<td>1.7 (0.02)</td>
<td>2.0 (0.03)</td>
<td>29 (9)</td>
<td>43 (6)</td>
<td>−40 (3)</td>
</tr>
<tr>
<td>Ir+Wc</td>
<td>1.7 (0.04)</td>
<td>2.0 (0.02)</td>
<td>16 (11)</td>
<td>35 (7)</td>
<td>39 (24)</td>
</tr>
<tr>
<td>Ir+Rc</td>
<td>1.7 (0.02)</td>
<td>2.0 (0.02)</td>
<td>16 (6)</td>
<td>46 (11)</td>
<td>−11 (12)</td>
</tr>
</tbody>
</table>
Monocrops had a significantly greater apparent natural affinity for Cd uptake compared with the Wc in 0% lime treatments (Fig. 1B). The differences in clover Cd in the current study may be partially related to differences in the rhizosphere architecture in young plants. Newly established Rc forms a deep, thick tap root, with many fine lateral roots, whereas Wc forms many finer roots during its initial establishment (Weaver and Bruner, 1926). A bulkier root system with greater root density in the 0% lime Rc mixed crops may have increased uptake of Cd by the neighboring plants from increasing Cd solubilization due to greater soil acidification than in the Wc mixed crops. However, changes in soil pH induced by the rhizosphere may have been too subtle to detect using the methods employed in this study. If plant Cd uptake was enhanced in the mixed crops that included Rc compared with those that included Wc, despite the lack of significant difference in soil pH, this may explain why forage Cd concentrations were higher in the mixed crops that included Rc compared with those that included Wc in the no lime treatment.

Although Cd uptake by plants is dependent on many factors including soil Cd concentrations and soil pH (Tudorcanu and Phillips, 2004), the acidifying effect of the clovers on the rhizosphere differs between species (McLay et al., 1997, Tang, 1997), and plant Cd uptake is also related to individual plant Cd uptake kinetics (Stritsis and Claassen, 2013). These species-specific factors should be considered in future studies to better understand the plant-specific regulators of Cd uptake.

### Effects of Liming on Cadmium Uptake in Mixed-Cropped Systems

The liming rates used in this experiment increased the soil pH (Fig. 1A). It was originally hypothesized that liming would reduce forage Cd concentrations. However, the effectiveness of lime to do this depends on both plant species and liming rates (Bolan et al., 2003b; Al Mamun et al., 2016). This is consistent with the results from the current study where forage Cd concentrations (both significantly and nonsignificantly) increased (as in the Ir and Ir+Wc treatments), decreased (as in the Pr, Rc, and Pr+Wc treatments), or
increased or decreased depending on the amount of lime (as in the Wc, Pr+Rc, and Ir+Rc treatments), with lime additions (Fig. 1B). Generally, liming was least effective at reducing forage Cd concentrations when Ir was included in the mixed crops and was most effective at reducing forage Cd with increased yield when Pr was included (Table 3), suggesting that the ryegrass species influenced the treatment response to liming.

Consistent with the original hypothesis that liming (both significantly and nonsignificantly, depending on the plant treatments) decreases the bioavailability of elements, most extractable elements decreased with increases in lime before the plants were added to the soil (Table 2). Liming can create soil conditions that reduce the bioavailability of Cd. Increases in pH can increase the binding of Cd with Fe or Mn oxides in soils (Chen et al., 2016), thereby inhibiting plant uptake of Cd (Mclaughlin et al., 1996; Loganathan et al., 2003). This occurs when liming increases the negative charge, or cation exchange capacity, in variable charge soils, which influences the soil's ability to hold essential nutrients and provides a buffer against soil acidification (Bolan et al., 1999) and can occur in the presence of N₂-fixing plants like clover (Tang, 1997).

There are a few factors that may have contributed to the differences in forage Cd in the Pr and Ir treatments at different liming rates (Fig. 1B). Experiments have shown that in solution, plants will take up increasing amounts of Cd with increasing concentrations of Cd (Page et al., 1972; Pettersson, 1976). The most likely explanation for the differences in forage Cd between the Pr and Ir response to liming may be attributed to plant-related differences in the exudates expelled from the root systems of the plants. The composition of root exudates varies by species (Marschner, 1995), and they can alter the soil microbial community in the rhizosphere (Sørensen, 1997). Other studies have noted that an exchange of cations can occur at the root surface when exudates are expelled. For example, forage Cd concentrations may be higher when roots release high concentrations of Ca, Mn, and Zn due to competition for adsorption sites on plant roots with other cations, which may contribute to the species-specific differences in adsorption of Cd (Jarvis et al., 1976; Vergara and Schalscha, 1992; Serrano et al., 2005; Liao and Selim 2009).

The contrasting responses of Pr (no change in forage Cd concentrations with increasing lime) and Ir (increasing forage Cd concentration with increasing lime) may be related to their N-uptake profiles; Ir takes up proportionally more N than Pr (Popay and Crush, 2010). This will create differences between the soil rhizosphere environments in the Pr and Ir treatments. This idea is supported by the correlation results (Table 4), whereby there were differences in the strength and direction of the relationship between elements in the treatments that include Pr and those that include Ir.

In some circumstances, liming can increase forage concentrations, as shown in the current experiment with higher Cd in Ir with higher lime treatments (Fig. 1B). Plant Cd uptake by roots may be increased under lime-induced Zn deficiency (Smolders, 2001). Some evidence of this is shown in the nonsignificant negative correlations between Cd and Zn in the Ir+Wc treatment (Table 4). The effects of lime-induced Zn deficiency on plant Cd uptake have not been directly considered in this study. Future studies should explore the role of plant-specific root exudates on plant Cd uptake to better understand the mechanisms by which various plant species take up Cd under various conditions.
Gray et al., 1999b; Loganathan et al., 2012. Other research tested the effects of different irrigation treatments (flooded for 3 d vs. not flooded) on extractable soil Cd concentrations and found no significant effect of these irrigation treatments on extractable soil Cd concentrations or on the soil pH within the same soil type, suggesting that soil Cd is insensitive to short-term periods of soil saturation (Stafford et al., 2018).

The increase in Mn concentrations in the 0% lime treatment before and after planting may be attributed to the slightly acidic pH (~5.11 before planting, Table 2); the dissolution Mn hydrous oxides—and therefore the release of Cd—can occur under oxidizing conditions when soil pH is mildly acidic (Gotoh and Patrick, 1972), suggesting that regardless of the water application to the soil during our experiment, there may have been increases in soil extractable Mn in the 0% lime treatment.

Conclusions
Ideally, liming at low rates could be used to reduce solubility and mobility of soil Cd in mixed clover and ryegrass pasture. However, the interactions between forage Cd uptake and the soil environment are complex and there was no consistent relationship observed between soil pH and Cd among the treatments in the current study. Many factors may have influenced the experimental outcomes including soil pH, solubility of Cd from liming, interaction between Cd and other soil nutrients, and plant root exudates and their impact on the soil environment and subsequently on forage Cd.

Liming at rates of 1 to 2% of the soil dry weight, which were used in this study, increased forage yields in all mixed-crop treatments compared with 0% lime. However, lime prevented increases in forage Cd in mixed-crop systems that included Pr (Pr+WC and Pr+RC) but increased forage Cd in treatments that included Ir, especially the Ir+WC treatment.

With the data available, we are not able to conclude what mechanisms were responsible for reduced forage Cd in the Pr, Wc, Rc, Pr+Wc, and Pr+Rc treatments associated with liming. However, the stark differences in forage Cd between the mixed crops that contain Pr and Ir in response to liming suggest that the results were due to a combination of Cd binding in soil and competition with other elements during plant uptake, which were induced by rhizosphere exudates.

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References


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