Published August 16, 2018

Journal of Environmental Quality

TECHNICAL REPORTS

TRACE ELEMENTS IN THE ENVIRONMENT

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 ($CdSO_4$ ~ 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 ($CdSO_4$) 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,

Copyright © American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. 5585 Guilford Rd., Madison, WI 53711 USA. All rights reserved.

J. Environ. Qual. 47:1249–1257 (2018)
doi:10.2134/jeq2018.01.0015
This is an open access article distributed under the terms of the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Received 10 Jan. 2018.
Accepted 9 July 2018.
*Corresponding author (brett.robinson@canterbury.ac.nz).

E. Benyas, J. Owens, and S. Seyedalikhani, Dep. of Soil and Physical Sciences, Faculty of Agriculture and Life Sciences, Lincoln Univ., PO Box 85084, Lincoln 7647, New Zealand; B. Robinson, School of Physical Sciences and Chemical Sciences, Univ. of Canterbury, Christchurch 8041, New Zealand. Assigned to Associate Editor Matthew Polizzotto.

Abbreviations: AgTU, silver thiourea; ICP–OES, inductively coupled plasma–optical emission spectrometer; Ir, Italian ryegrass; Pr, perennial ryegrass; Rc, red clover; Wc, white clover.

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 (Rc, *T. pratense* L.) (Frame and Newbould, 1986; Brock and Hay, 1996; Ramírez-Restrepo and Barry, 2005; Sanderson et al., 2005). Italian ryegrass (Ir, *Lolium multiflorum* Lam.) may be increasingly used in grazed pastures due to its potential to reduce NO_3 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 soil mass), and one of eight plant treatments. The plant treatments were monocrops of perennial ryegrass (Pr), Italian ryegrass (Ir), white clover (Wc), or red clover (Rc), or ryegrass–clover mixed crops of Pr+Wc, Pr+Rc, Ir+Wc, or Ir+Rc.

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 $CdSO_4$ 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 $CdSO_4$ solution was added to soil to ensure that it contained sufficient Cd concentrations for the experiment (Table 2).

Lime Additions

Lime (solid limestone $CaCO_3$, 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 $CdSO_4$ 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.

Properties	Value
Clay/silt/sand (%)†	4/20/76
pH (H_2O)†	5.1
Cation exchange capacity ($cmol_c$ kg^{-1})	12.3
Soil C (%)†	3.3
Soil N (%)†	0.3
Cd (mg kg^{-1})	1.31
Al (mg kg^{-1})	24,720
Cu (mg kg^{-1})	4.65
Fe (g kg^{-1})	12.4
K (g kg^{-1})	4.75
Mg (g kg^{-1})	3.86
Mn (mg kg^{-1})	369
Na (mg kg^{-1})	338
P (mg kg^{-1})	718
Pb (mg kg^{-1})	12.6
S (mg kg^{-1})	336
Zn (mg kg^{-1})	61.7
Ca (g kg^{-1})	3.44

† These properties were taken from previous research using the same soil as was used in this research (Simmler et al., 2013)

Table 2. Extractable soil chemical elements before planting and after planting in the perennial ryegrass monocrop for each lime treatment.

Extractable concentration	Before planting						After harvesting					
	0% liming		1% liming		2% liming		0% liming		1% liming		2% liming	
	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE	Mean	±SE
Cd (mg kg^{-1})	0.196	0.012	0.013	0.003	0.003	0.000	0.190	0.014	0.005	0.001	0.003	0.001
Al (mg kg^{-1})	12.85	0.342	0.373	0.004	0.322	0.006	11.56	0.650	0.318	0.008	0.321	0.017
Cu (mg kg^{-1})	0.034	0.003	0.021	0.001	0.020	0.001	0.013	0.002	0.020	0.002	0.020	0.003
Fe (mg kg^{-1})	0.753	0.022	0.296	0.002	0.264	0.005	0.855	0.055	0.278	0.008	0.278	0.011
K (mg kg^{-1})	40.61	0.820	33.96	0.207	34.58	0.153	24.14	1.098	22.42	0.953	19.08	0.770
Mg (mg kg^{-1})	144.20	1.581	122.70	1.315	85.83	0.778	135.29	0.820	89.58	0.334	69.56	0.746
Mn (mg kg^{-1})	23.85	1.258	2.967	0.048	1.093	0.034	34.54	3.272	3.773	1.038	4.232	0.884
Na (mg kg^{-1})	80.36	2.233	77.34	0.674	70.68	2.956	101.23	5.768	79.10	3.213	77.22	6.080
Ni (mg kg^{-1})	0.046	0.005	–	–	–	–	0.024	0.003	–	–	0.009	0.006
P (mg kg^{-1})	2.083	0.079	1.946	0.007	2.492	0.086	2.008	0.047	1.907	0.068	2.030	0.122
Pb (mg kg^{-1})	–	–	–	–	–	–	–	–	0.012	–	0.008	0.001
S (mg kg^{-1})	17.51	2.573	26.23	1.034	33.09	2.484	14.65	1.056	13.10	0.675	13.20	0.668
Zn (mg kg^{-1})	1.625	0.114	0.536	0.048	0.658	0.080	1.348	0.099	0.477	0.063	0.335	0.084
pH	5.11	0.044	6.68	0.013	7.03	0.006	–	–	–	–	–	–
Soil N (%)	0.285	0.008	0.277	0.002	0.264	0.004	–	–	–	–	–	–
Soil C (%)	3.19	0.079	3.27	0.026	3.33	0.032	–	–	–	–	–	–
Soil C/N ratio	11.19	0.042	11.81	0.048	12.63	0.312	–	–	–	–	–	–

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 removal 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 HNO_3 and 1 mL 30% H_2O_2 (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 HNO_3 and 1 mL of H_2O_2 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 $Ca(NO_3)_2$ extracts on the ICP–OES. Dried soil (0.5 g) was weighted into a 50-mL tube, and 30 mL of 0.05 M $Ca(NO_3)_2$ 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 (mg 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 $P \leq 0.05$.

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 ryegrass treatments (Fig. 1A). This is contrary to the original hypothesis that the addition of clovers to ryegrasses would decrease soil pH.

Plant treatments 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 soil without lime (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 liming 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 plants 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

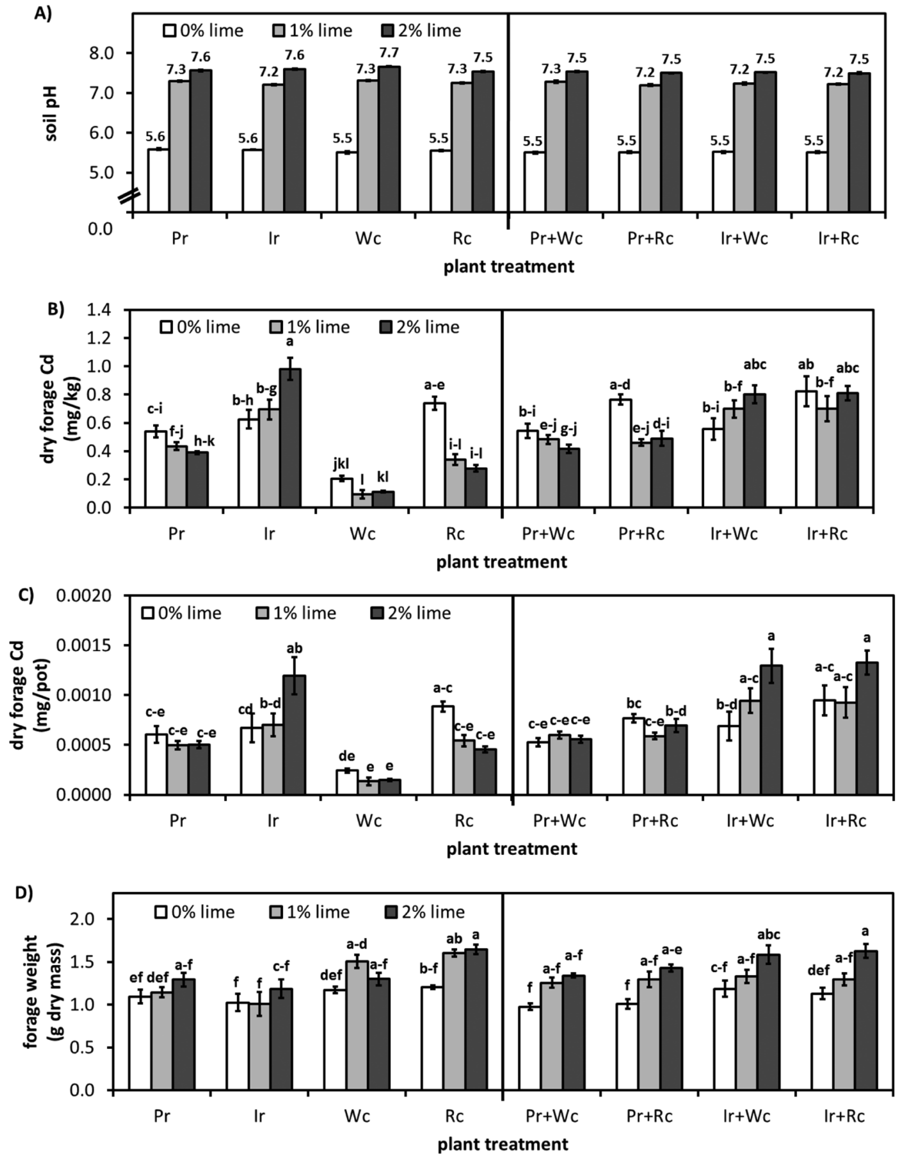

Fig. 1. (A) The soil pH (with the pH values above the bars), (B) dry forage Cd, (C) forage yield, and (D) forage weight for all lime and plant treatments where Pr is perennial ryegrass, Ir is Italian ryegrass, Wc is white clover, Rc is red clover, Pr+Wc is perennial ryegrass and white clover, Pr+Rc is perennial ryegrass and red clover, Ir+Wc is Italian ryegrass and white clover, and Ir+Rc is Italian ryegrass and red clover. Bars with different letters are significantly different at $P \leq 0.05$ as determined by the Tukey's test.

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.

Plant treatment	Difference between lime treatments							
	Soil pH		Forage weight		Cd dry forage concentration		Cd dry forage concentration	
	Lime treatments							
	0 vs. 1%	0 vs. 2%	0 vs. 1%	0 vs. 2%	0 vs. 1%	0 vs. 2%	0 vs. 1%	0 vs. 2%
			g dry matter		mg kg^{-1}		mg pot^{-1}	
Pr	1.7 (0.02)	2.0 (0.03)	8 (11)	22 (14)	−15 (12)	−25 (7)	−5 (21)	−7 (15)
Ir	1.6 (0.01)	2.0 (0.02)	5 (21)	21 (17)	22 (23)	64 (22)	37 (39)	114 (63)
Wc	1.8 (0.03)	2.2 (0.02)	30 (9)	12 (8)	−51 (18)	−42 (7)	−40 (20)	−36 (8)
Rc	1.7 (0.02)	2.0 (0.02)	33 (4)	37 (6)	−52 (7)	−61 (5)	−36 (10)	−47 (6)
Pr+Wc	1.8 (0.02)	2.0 (0.02)	29 (6)	38 (6)	−10 (5)	−19 (11)	16 (9)	11 (15)
Pr+Rc	1.7 (0.02)	2.0 (0.03)	29 (9)	43 (6)	−40 (3)	−35 (8)	−23 (3)	−6 (16)
Ir+Wc	1.7 (0.04)	2.0 (0.02)	16 (11)	35 (7)	39 (24)	62 (31)	71 (41)	126 (52)
Ir+Rc	1.7 (0.02)	2.0 (0.02)	16 (6)	46 (11)	−11 (12)	6 (13)	6 (18)	57 (23)

Table 4. The *r* values for Pearson correlations between selected plant variables and soil pH from all lime treatments for perennial ryegrass (Pr), Italian ryegrass (Ir), white clover (Wc), red clover (Rc), and mixed-crop treatments.

Variable	*r* value for Pearson correlation with plant Cd (mg kg^{-1})							
	Plant treatment							
	Pr	Ir	Wc	Rc	Pr+Wc	Pr+Rc	Ir+Wc	Ir+Rc
Plant dry wt.	−0.02	0.54*	−0.62*	−0.89*	0.24	−0.41	0.34	0.24
Plant fresh wt.	−0.17	0.62*	−0.64*	−0.89*	0.33	−0.45	0.40	0.33
Plant Zn	0.39	0.56*	0.52*	0.88*	0.38	0.07	−0.27	0.38
Plant Cu	−0.69*	0.49*	0.42	0.79*	0.06	0.03	0.01	0.06
Plant Fe	0.22	0.67*	0.17	0.58*	0.18	0.05	0.18	0.18
Plant Mn	0.50*	0.16	0.74*	0.90*	−0.19	0.45	−0.50*	−0.19
Plant B	0.66*	0.56*	−0.18	0.42	−0.12	0.21	−0.14	−0.12
Plant K	−0.09	0.48*	0.60*	0.91*	0.26	0.15	−0.14	0.26
Plant P	−0.22	0.35	−0.64*	−0.70*	0.05	−0.12	−0.43	0.05
Plant Mg	0.10	0.75*	0.07	−0.44	0.17	−0.06	0.13	0.17
Plant Ca	−0.30	0.78*	−0.36	−0.51*	0.32	−0.08	−0.05	0.32
Plant Na	−0.21	0.69*	−0.13	0.06	0.29	−0.09	0.43	0.29
Plant N (%)	−0.41	0.19	−0.52*	−0.35	0.23	−0.23	0.21	0.23
Plant C (%)	0.25	0.29	0.02	−0.37	0.17	−0.28	0.47	0.17
Plant C/N	0.46	−0.23	0.58*	0.27	−0.22	0.25	−0.17	−0.22
Soil pH	−0.69*	0.54*	−0.69*	−0.92*	0.17	−0.32	0.01	0.17

* Significant at the 0.05 probability level.

(Fig. 1B). Lime induced increases in soil pH tended to increase forage yield (Fig. 1D). However, the interaction between the presence of the clovers and the application of lime in the mixed-crops failed to consistently lower forage Cd concentrations.

Effects of Mixing Clovers and Ryegrasses on Forage Cadmium Concentrations

Failure to consistently observe higher forage Cd concentrations in mixed clover–ryegrass systems compared with the ryegrass monocrops at 0% lime, as originally hypothesized, differs from the results of other studies. Higher Cd concentrations have been noted in various crops mixed with Japanese clover [*Kummerowia striata* (Thunb.) Schindl.], compared with monocrops of the same plants (An et al., 2011, Liu et al., 2012). Forage Cd concentrations in the mixed-crop species increased due to soil acidification from N fixation by clovers. A net efflux of H^+ ions into the soil increases clover cation uptake (Tang, 1997). The small differences in soil pH (~0.1–0.2 units) between the monocrop ryegrasses and different mixed crops in the lime-free treatments (Fig. 1A) may be partially attributed to the similar conditions in which the plants were grown. The magnitude of rhizosphere soil acidification under clovers is influenced by nutrient availability, growth conditions, and the stage of plant growth (Pierre and Banwart, 1973). With only two clover species used during this experiment, and all of the plants being the same age, grown under the same conditions, and grown in soils with the same concentrations of Cd, the abovementioned factors known to contribute to variability in rhizosphere pH were minimized.

There were some significant differences in forage Cd concentrations in the mixed-cropped systems that appeared to be related to clover species. Specifically, in the 0% lime treatment, there tended to be (nonsignificantly) higher forage Cd from the mixed crops that contained Rc (Pr+Rc and Ir+Rc) compared with those that contained Wc (Pr+Wc and Ir+Wc) (Fig. 1B). This suggests the influence of factors other than soil pH. The Rc 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 (Tudoreanu 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 N_2–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 difference 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.

Compound Effects of Liming and Plant Species on Forage Yield and Cadmium Concentration

Consistent with the original hypothesis, liming (both significantly and nonsignificantly) increased the forage yield in both mono- and mixed-crop systems (Fig. 1C). In the mixed crops, liming at 2% had a greater effect on forage yield when the treatments included Rc compared with treatments that included Wc (Fig. 1C). However, liming as a strategy to negate the uptake of Cd by forage while also increasing forage yields was most effective in treatments that included Pr (Fig. 1B).

The mass of Cd per pot shows how differences in forage yield and forage Cd concentrations combine to affect the potential intake of Cd by grazing animals. Although we have not identified where in our forage the Cd was stored, these results suggest Pr mixed crops and monocrops present the lowest potential intake of Cd by grazers (Fig. 1C). Although more research is needed to further understand the mechanisms that regulate Cd uptake by different plant species, it can be concluded that pH in isolation is not suitably variable for determining plant uptake of Cd when pasture species are combined in a system. The outcome from the current study that greater liming increased forage yields but did not consistently decrease forage Cd (Fig. 1B) is an important finding in the development of grazing management strategies. The results suggest that a mixture of Ir and clovers is a poor choice as grazing forage in Cd-contaminated soils.

Italian ryegrass is likely to increase in popularity as a pasture species, as it is well suited for helping reduce NO_3 leaching as drainage (Malcolm et al., 2015; Woods et al., 2016; Bryant et al., 2018). However, the results from our study suggest that it may not be a suitable pasture species for grazing in Cd-contaminated soil due to its high uptake of Cd under the tested conditions. Using Ir as a grazed pasture species could result in trading one problem (NO_3 leaching) for another (higher Cd consumption by grazing cattle).

Pastures that included Pr+Wc and Pr+Rc had the most favorable results as a foraging crop. These species combinations increased forage yield without increasing forage Cd. Optimization of grazing forage for the highest yield with the lowest Cd concentrations will entail a greater understanding of the mechanisms of Cd uptake. Other measures of nutritional value (i.e., crude protein, detergent fiber, etc.) were not measured during this study but should be considered in future studies, since they will factor into the choice of forage.

Experimental Design Considerations

There is a chance that water additions during our experiment may have affected the soil redox, thereby influencing the availability of soluble Cd. Soils under prolonged saturation and strongly reducing conditions form insoluble Cd complexes, which decreases Cd availability (de Livera et al., 2011; Zhang et al., 2012; Zheng et al., 2013). Instead, there is some evidence (increases in Fe and Mn soil concentrations from 0% lime before and after the experiment, Table 2) that short periods of saturation after water additions may have temporarily created weakly reducing soil conditions. These conditions can develop quickly (Ponnamperuma, 1972). Such mild and temporarily reducing conditions have the potential to influence solubility of Cd; Cd will be released if was sorbed to Fe and Mn hydrous oxides and dissolution occurs (Kim and Fergusson, 1992; Backes et al., 1995;

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 forage 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.

Acknowledgments

We gratefully acknowledge the Centre for Integrated Biowaste Research (CIBR) for partially funding this project.

References

Al Mamun, S., G. Chanson, E. Muliadia, M. Benyas, N. Aktar, R.C. Lehto, et al. 2016. Municipal composts reduce the transfer of Cd from soil to vegetables. Environ. Pollut. 213:8–15. doi:10.1016/j.envpol.2016.01.072

An, L., Y. Pan, Z. Wang, and C. Zhu. 2011. Heavy metal absorption status of five plant species in monoculture and intercropping. Plant Soil 345:237–245. doi:10.1007/s11104-011-0775-1

Backes, C.A., R.G. McLaren, A.W. Rate, and R.S. Swift. 1995. Kinetics of cadmium and cobalt desorption from iron and manganese oxides. Soil Sci. Soc. Am. J. 59:778–785. doi:10.2136/sssaj1995.03615995005900030021x

Black, A., R. McLaren, S. Reichman, T. Speir, and L. Condron. 2012. Examining the integrity of soil metal bioavailability assays in the presence of organic amendments to metal-spiked soils. Soil Use Manage. 28:89–100. doi:10.1111/j.1475-2743.2011.00384.x

Blakemore, L., P. Searle, and B. Daly. 1987. Methods for chemical analysis of soils. Sci. Rep. N. Z. Soil Bureau, Lower Hutt, New Zealand.

Bolan, N.S., D.C. Adriano, P. Duraisamy, and A. Mani. 2003a. Immobilization and phytoavailability of cadmium in variable charge soils. III. Effect of biosolid compost addition. Plant Soil 256:231–241. doi:10.1023/A:1026288021059

Bolan, N.S., R. Naidu, J. Syers, and R. Tillman. 1999. Surface charge and solute interactions in soils. Adv. Agron. 67:87–140. doi:10.1016/S0065-2113(08)60514-3

Bolan, N.S., R. Nanthi, and V.P. Duraisamy. 2003b. Role of inorganic and organic soil amendments on immobilisation and phytoavailability of heavy metals: A review involving specific case studies. Soil Res. 41:533–555. doi:10.1071/SR02122

Brock, J., and M. Hay. 1996. A review of the role of grazing management on the growth and performance of white clover cultivars in lowland New Zealand pastures. Publ. no. 11. Grassl. Res. Practice Ser. 6. Agron. Soc. N. Z., New Zealand.

Bryant, R., B. Welten, D. Costall, P. Shorten, and G. Edwards. 2018. Milk yield and urinary-nitrogen excretion of dairy cows grazing forb pasture mixtures designed to reduce nitrogen leaching. Livest. Sci. 209:46–53. doi:10.1016/j.livsci.2018.01.009

Cadmium Working Group. 2011. Cadmium and New Zealand agriculture and horticulture: A strategy for long term risk management. Tech. Paper 2011/02. Minist. Agric. For., Wellington, New Zealand.

Chen, Y., T. Xie, Q. Liang, M. Liu, M. Zhao, M. Wang, and G. Wang. 2016. Effectiveness of lime and peat applications on cadmium availability in a paddy soil under various moisture regimes. Environ. Sci. Pollut. Res. Int. 23:7757–7766. doi:10.1007/s11356-015-5930-4

de Livera, J., M.J. McLaughlin, G.M. Hettiarachchi, J.K. Kirby, and D.G. Beak. 2011. Cadmium solubility in paddy soils: Effects of soil oxidation, metal sulfides and competitive ions. Sci. Total Environ. 409:1489–1497. doi:10.1016/j.scitotenv.2010.12.028

Fay, D. 2007. Soil geochemical atlas of Ireland. Teagasc and Environ. Prot. Agency, Carlow, Ireland.

Frame, J., and P. Newbould. 1986. Agronomy of white clover. Adv. Agron. 40:1–88. doi:10.1016/S0065-2113(08)60280-1

Gotoh, S., and W. Patrick. 1972. Transformation of manganese in a waterlogged soil as affected by redox potential and pH. Soil Sci. Soc. Am. J. 36:738–742. doi:10.2136/sssaj1972.03615995003600050018x

Grant, C.A., L.D. Bailey, M.J. McLaughlin, and B.R. Singh. 1999. Management factors which influence cadmium concentrations in crops. In: M.J. McLaughlin and B.R. Singh, editors, Cadmium in soils and plants. Springer, Dordrecht, the Netherlands, p. 151–198. doi:10.1007/978-94-011-4473-5_7

Gray, C., R. McLaren, A. Roberts, and L. Condron. 1999a. Cadmium phytoavailability in some New Zealand soils. Soil Res. 37:461–478. doi:10.1071/S98070

Gray, C., R. McLaren, A. Roberts, and L. Condron. 1999b. Solubility, sorption and desorption of native and added cadmium in relation to properties of soils in New Zealand. Eur. J. Soil Sci. 50:127–137. doi:10.1046/j.1365-2389.1999.00221.x

Hewitt, A.E. 2010. New Zealand soil classification. Manaaki-Whenua Press, Lincoln, New Zealand.

Jarvis, S., L. Jones, and M. Hopper. 1976. Cadmium uptake from solution by plants and its transport from roots to shoots. Plant Soil 44:179–191. doi:10.1007/BF00016965

Kabata-Pendias, A., and A.B. Mukherjee. 2007. Trace elements from soil to human. Springer Verlag, Berlin. doi:10.1007/978-3-540-32714-1

Kim, N., and J. Fergusson. 1992. Adsorption of cadmium by an aquent New Zealand soil and its components. Soil Res. 30:159–167. doi:10.1071/SR9920159

Landcare Research. 2018. Soil order. N. Z. Soils Portal. https://soils.landcareresearch.co.nz/index.php/describing-soils/nzsc/soil-order (accessed 8 Mar. 2018).

Latati, M., D. Blavet, N. Alkama, H. Laoufi, J.J. Drevon, F. Gérard, et al. 2014. The intercropping cowpea-maize improves soil phosphorus availability and maize yields in an alkaline soil. Plant Soil 385:181–191. doi:10.1007/s11104-014-2214-6

Lee, J., J. Rounce, A. Mackay, and N. Grace. 1996. Accumulation of cadmium with time in Romney sheep grazing ryegrass-white clover pasture: Effect of cadmium from pasture and soil intake. Aust. J. Agric. Res. 47:877–894. doi:10.1071/AR9960877

Li, N.Y., Z.A. Li, P. Zhuang, B. Zou, and M. McBride. 2009. Cadmium uptake from soil by maize with intercrops. Water Air Soil Pollut. 199:45–56. doi:10.1007/s11270-008-9858-x

Liao, L., and H. Selim. 2009. Competitive sorption of nickel and cadmium in different soils. Soil Sci. 174:549–555. doi:10.1097/SS.0b013e3181bbbd27

Liu, L., Q. Zhang, L. Hu, J. Tang, L. Xu, X. Yang, et al. 2012. Legumes can increase cadmium contamination in neighboring crops. PLoS One 7:e42944. doi:10.1371/journal.pone.0042944

Liu, M., Z.P. Li, and X.C. Zhai. 2013. Peanut straw decomposition products promoted by chemical additives and their effect on enzymatic activity and microbial functional diversity in red soil. Compost Sci. Util. 21:76–86. doi:10.1080/1065657X.2013.829674

Loganathan, P., M.J. Hedley, N. Grace, J. Lee, S. Cronin, N.S. Bolan, and J. Zanders. 2003. Fertiliser contaminants in New Zealand grazed pasture with special reference to cadmium and fluorine: A review. Soil Res. 41:501–532. doi:10.1071/SR02126

Loganathan, P., S. Vigneswaran, J. Kandasamy, and R. Naidu. 2012. Cadmium sorption and desorption in soils: A review. Crit. Rev. Environ. Sci. Technol. 42:489–533. doi:10.1080/10643389.2010.520234

López-Chuken, U.J., and S.D. Young. 2010. Modelling sulphate-enhanced cadmium uptake by Zea mays from nutrient solution under conditions of constant free Cd(2+) ion activity. J. Environ. Sci. (China) 22:1080–1085. doi:10.1016/S1001-0742(09)60220-5

Mahler, R.J., J. Ryan, and T. Reed. 1987. Cadmium sulfate application to sludge-amended soils I. Effect on yield and cadmium availability to plants. Sci. Total Environ. 67:117–131. doi:10.1016/0048-9697(87)90205-1

Malcolm, B.J., J.L. Moir, K.C. Cameron, H.J. Di, and G.R. Edwards. 2015. Influence of plant growth and root architecture of Italian ryegrass (*Lolium multiflorum*) and tall fescue (*Festuca arundinacea*) on N recovery during winter. Grass Forage Sci. 70:600–610. doi:10.1111/gfs.12157

Marschner, H. 1995. Mineral nutrition of higher plants. Academic Press, London.

Mclaughlin, M., K. Tiller, R. Naidu, and D. Stevens. 1996. Review: The behaviour and environmental impact of contaminants in fertilizers. Soil Res. 34:1–54. doi:10.1071/SR9960001

McLay, C.D.A., L. Barton, and C. Tang. 1997. Acidification potential of ten grain legume species grown in nutrient solution. Aust. J. Agric. Res. 48:1025–1032. doi:10.1071/A96174

Mortvedt, J.J. 1995. Heavy metal contaminants in inorganic and organic fertilizers. Fert. Res. 43:55–61. doi:10.1007/BF00747683

Page, A., F. Bingham, and C. Nelson. 1972. Cadmium absorption and growth of various plant species as influenced by solution cadmium concentration. J. Environ. Qual. 1:288–291. doi:10.2134/jeq1972.00472425000100030017x

Pettersson, O. 1976. Heavy-metal ion uptake by plants from nutrient solutions with metal ion, plant species and growth period variations. Plant Soil 45:445–459.

Pierre, W.H., and W.L. Banwart. 1973. Excess-base and excess-base/nitrogen ratio of various crop species and parts of plants. Agron. J. 65:91–96. doi:10.2134/agronj1973.00021962006500010028x

Ponnamperuma, F.N. 1972. The chemistry of submerged soils. Adv. Agron. 24:29–96.

Popay, A.J., and J.R. Crush. 2010. Influence of different forage grasses on nitrate capture and leaching loss from a pumice soil. Grass Forage Sci. 65:28–37.

R Core Team. 2013. R: A language and environment for statistical computing. R Found. Stat. Comput., Vienna.

Ramírez-Restrepo, C., and T. Barry. 2005. Alternative temperate forages containing secondary compounds for improving sustainable productivity in grazing ruminants. Anim. Feed Sci. Technol. 120:179–201. doi:10.1016/j.anifeedsci.2005.01.015

Roberts, A.H.C., and R.D. Longhurst. 2002. Cadmium cycling in sheep-grazed hill-country pastures. N. Z. J. Agric. Res. 45:103–112. doi:10.1080/00288233.2002.9513499

Robinson, B.H., G. Bañuelos, H.M. Conesa, M.W. Evangelou, and R. Schulin. 2009. The phytomanagement of trace elements in soil. Crit. Rev. Plant Sci. 28:240–266. doi:10.1080/07352680903035424

Sanderson, M., K. Soder, L. Muller, K. Klement, R. Skinner, and S. Goslee. 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. 97:1465–1471. doi:10.2134/agronj2005.0032

Serrano, S., F. Garrido, C. Campbell, and M. Garcıa-González. 2005. Competitive sorption of cadmium and lead in acid soils of Central Spain. Geoderma 124:91–104. doi:10.1016/j.geoderma.2004.04.002

Simmler, M., L. Ciadamidaro, R. Schulin, P. Madejoìn, R. Reiser, L. Clucas, et al. 2013. Lignite reduces the solubility and plant uptake of cadmium in pasturelands. Environ. Sci. Technol. 47:4497–4504. doi:10.1021/es303118a

Smolders, E. 2001. Cadmium uptake by plants. Int. J. Occup. Med. Environ. Health 14:177–183.

Sørensen, J. 1997. The rhizosphere as a habitat for soil microorganisms. In: J.D. Van Elsas, et al., editors, Modern microbiology. Marcel Dekker, New York. p. 21–45.

SPSS. 2007. SPSS for Windows. Release 16.0. SPSS, Chicago, IL.

Stafford, A., P. Jeyakumar, M. Hedley, and C. Anderson. 2018. Influence of soil moisture status on soil cadmium phytoavailability and accumulation in plantain (*Plantago lanceolata*). Soil Syst. 2:9. doi:10.3390/soils2010009

Street, J.J., B. Sabey, and W. Lindsay. 1978. Influence of pH, phosphorus, cadmium, sewage sludge, and incubation time on the solubility and plant uptake of cadmium. J. Environ. Qual. 7:286–290. doi:10.2134/jeq1978.00472425000700020027x

Stritsis, C., and N. Claassen. 2013. Cadmium uptake kinetics and plants factors of shoot Cd concentration. Plant Soil 367:591–603. doi:10.1007/s11104-012-1498-7

Tang, C. 1997. Soil acidification under legumes: A review. In: D. Williamson, editor, Proceedings of the 4th Triennial Western Australian Soil Science Conference, Geraldton, WA. 30 Sept.–2 Oct. 1997. Aust. Soc. Soil Sci. (WA Branch), Geraldton. p. 121–127.

Tudoreanu, L., and C.J.C. Phillips. 2004. Empirical models of cadmium accumulation in maize, rye grass and soya bean plants. J. Sci. Food Agric. 84:845–852. doi:10.1002/jsfa.1730

Vergara, I., and E. Schalscha. 1992. Cadmium-copper competitive absorption in soils. Agrochimica, Bolzano, Italy.

Weaver, J. E., and W. E. Bruner. 1926. Root development of field crops. Botanical Gazette 82:228.

Welch, R.M., and W.A. Norvell. 1999. Mechanisms of cadmium uptake, translocation and deposition in plants. In: M.J. McLaughlin and B.R. Singh, editors, Cadmium in soils and plants. Springer, Dordrecht, the Netherlands. p. 125–150. doi:10.1007/978-94-011-4473-5_6

Woods, R., K. Cameron, G. Edwards, H. Di, and T. Clough. 2016. Effects of forage type and gibberellic acid on nitrate leaching losses. Soil Use Manage. 32:565–572. doi:10.1111/sum.12297

Zhang, C., Y. Ge, H. Yao, X. Chen, and M. Hu. 2012. Iron oxidation-reduction and its impacts on cadmium bioavailability in paddy soils: A review. Front. Environ. Sci. Eng. 6:509–517. doi:10.1007/s11783-012-0394-y

Zheng, S., X. Zheng, and C. Chen. 2013. Transformation of metal speciation in purple soil as affected by waterlogging. Int. J. Environ. Sci. Technol. 10:351–358. doi:10.1007/s13762-012-0146-3