Plant acquisition and metabolism of the synthetic nitrification inhibitor dicyandiamide and naturally-occurring guanidine from agricultural soils
Marsden, K.A.; Scowen, M.; Hill, P.W.; Jones, D.L.; Chadwick, D.R.

Plant and Soil

DOI:
10.1007/s11104-015-2549-7

Published: 01/10/2015

Publisher's PDF, also known as Version of record

Cyswllt i'r cyhoeddiad / Link to publication

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):
Marsden, K. A., Scowen, M., Hill, P. W., Jones, D. L., & Chadwick, D. R. (2015). Plant acquisition and metabolism of the synthetic nitrification inhibitor dicyandiamide and naturally-occurring guanidine from agricultural soils. Plant and Soil, 395(1), 201-214. https://doi.org/10.1007/s11104-015-2549-7

Hawliau Cyffredinol / General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
Plant acquisition and metabolism of the synthetic nitrification inhibitor dicyandiamide and naturally-occurring guanidine from agricultural soils

Karina A. Marsden · Matthew Scowen · Paul W. Hill · Davey L. Jones · David R. Chadwick

Received: 16 March 2015 / Accepted: 2 June 2015 © The Author(s) 2015. This article is published with open access at Springerlink.com

Abstract
Background and aims There is increasing interest and use of nitrification inhibitors (NI) in agroecosystems, yet little is known of their fate in planta. Residues of the organic, N-rich NI, dicyandiamide (DCD), have been found in milk products following commercial application to pasture. We investigated whether plant acquisition and metabolism of DCD were consistent with plant-mediated transmission from soil to agricultural food products.

Methods Uptake rates, translocation to the shoot, degradation of the label within wheat tissue and availability within two soils of DCD and the structurally similar naturally occurring N-rich molecule, guanidine, were measured using \(^{14}\)C labelling.

Results Under sterile conditions, over 2 h wheat took up (34 and 14 μmol g\(^{-1}\) root DW h\(^{-1}\) at 1 mM: DCD and guanidine, respectively), translocated (7–15 and 19–22 %) and metabolised (0.4 and 0.9 % of uptake) DCD- and guanidine-\(^{14}\)C. Both molecules were also acquired from soil by wheat despite concurrent soil sorption and microbial uptake.

Conclusions Both DCD and guanidine can be acquired and metabolised by graminaceous plants. Although probably not a significant route of N acquisition, plant uptake provides a direct route of DCD entry into the food chain.

Keywords Bioavailability · Dicyandiamide (DCD) · Guanidine · Mineralization · Nitrification inhibitor · Nitrogen cycle

Introduction
Within agricultural soils, nitrification represents one of the dominant nitrogen (N) flow pathways and is responsible for generating NO\(_3^-\) which can be lost to the wider environment via leaching and denitrification (Zerulla et al. 2001). To reduce these N losses, effective management strategies are required to improve N use efficiency (NUE) within most agroecosystems. One potential solution to the problem is the application of synthetic or natural nitrification inhibitors (NI) to the soil to slow the conversion of NH\(_4^+\) to NO\(_3^-\) (Subbarao et al. 2012; Abalos et al. 2014).

Among the many identified NI, synthetic dicyandiamide (DCD; C\(_2\)H\(_4\)N\(_4\)) is one of the most widely researched and one of the few used at a commercial scale (O’Callaghan et al. 2010; Liu et al. 2013). DCD has been investigated in a wide range of arable and livestock-based agroecosystems, where applications of DCD (10–30 kg ha\(^{-1}\)) have been shown to be effective in reducing nitrous oxide (N\(_2\)O) emissions following spreading of either N fertilisers (Weiske et al. 2001; Di and Cameron 2006; Cui et al. 2011), livestock slurry (Hatch et al. 2005) or ruminant urine (Di and Cameron...
2006; Dai et al. 2013; Barneze et al. 2015). DCD application to soil has also been shown to reduce NO$_3^-$ leaching after the application of inorganic N fertilisers and from livestock urine patches (Di et al. 2009; Cui et al. 2011). A recent meta-analysis investigating the effect of soil-applied NI (including DCD), indicated that on average they result in an 8 % increase in crop yield and a 13 % increase in NUE (Abalos et al. 2014).

A range of application routes for DCD have been investigated including introduction to urine patches by oral administration to cattle (O’Connor et al. 2013; Welten et al. 2013), infusion of DCD into the rumen or abomasum of sheep (Ledgard et al. 2008), incorporation into fertiliser granules or addition of DCD in a biodegradable hydrogel to slow its release in soil (Minet et al. 2013). However, the simplest, least controversial and consequently most widespread route is direct application to soil. Although direct application to soil is both practical and has demonstrable efficacy, this method leaves DCD susceptible to degradation by soil microbes, which may reduce persistence and increases in NUE. There is also the potential for removal from soil due to uptake by plant roots.

In 2012, low level residues of DCD were found in New Zealand dairy products from which plant interception and uptake of DCD with subsequent transfer to ruminant milk have been hypothesised to be key vectors in its contamination (Kim et al. 2012; Chen et al. 2014). Although DCD is not currently known to pose a significant risk to human health, the discovery of DCD in milk has led to a voluntary suspension of sale and use of DCD in New Zealand until international acceptable limits for its presence in milk products can be agreed (Ministry for Primary Industries 2013).

We aimed to test the following hypotheses: 1) graminaceous plants have the capacity to take up both DCD and guanidine with their roots; 2) both DCD and guanidine can be metabolised by plants; 3) plants can take up both DCD and guanidine from soil; 4) the magnitude of competing substrate removal processes from soil (microbial uptake, mineralization and sorption) will be regulated by soil type.

Materials and methods

Soil properties

Two contrasting UK agricultural soils were used in this study (Table 1). The first was a mineral sandy loam textured Eutric Cambisol collected from a sheep-grazed fertilised grassland in North Wales (53°14′N, 4°01′W), while the second was an organic Sapric Histosol collected from an intensive arable production

---

**Fig. 1** Molecular structure of the nitrification inhibitor, dicyandiamide (DCD; Panel A), and its naturally occurring analogue guanidine (Panel B), which are used in this study.
Table 1  Soil properties of Eutric Cambisol and Sapric Histosol used in soil rhizosphere microcosms. Values represent means±SEM, n=4, letters indicate significant differences between the two soils and results are reported on a dry soil weight basis.

| Soil Property                  | Eutric Cambisol | Sapric Histosol |
|-------------------------------|----------------|-----------------|
| Moisture Content (%)          | 25.0±0.27 a    | 61.2±0.09 b     |
| Organic Matter (%)            | 7.70±1.41 a    | 77.2±0.47 b     |
| Cation Exchange Capacity      | 14.8±0.68a     | 80.8±0.80 b     |
| (meq 100 g⁻¹)                 | (0.2 μmol cm⁻³)|                 |
| pH                            | 6.67±0.14 a    | 6.37±0.06 a     |
| Electrical Conductivity (μS cm⁻¹)| 50.1±1.85 a    | 103±9.56 b     |
| Total Carbon (g kg⁻¹)         | 23.8±1.50 a    | 391±1.15 b      |
| Total Nitrogen (g kg⁻¹)       | 2.92±0.07 a    | 26.5±0.07 b     |
| Total Organic Carbon (mg C kg⁻¹)| 88.2±6.29 a    | 959±79.1 b     |
| Total Organic Nitrogen (mg N kg⁻¹)| 10.6±3.70 a    | 37.8±5.35 b     |
| Microbial C (g kg⁻¹)          | 1.58±0.05 a    | 4.41±0.20 b     |
| Microbial N (g kg⁻¹)          | 0.33±0.03 a    | 0.96±0.08 b     |
| NH₄⁺ (mg N kg⁻¹)              | 4.29±0.32 a    | 5.90±0.69 a     |
| NO₃⁻ (mg N kg⁻¹)              | 4.34±0.20 a    | 27.8±2.45 b     |
| PO₄³⁻ (mg P kg⁻¹)             | 9.98±0.39 a    | 32.0±6.68 b     |
| K⁺ (meq kg⁻¹)                 | 12.3±2.36 a    | 8.46±2.72 a     |
| Na⁺ (meq kg⁻¹)                | 0.95±0.05 a    | 5.08±0.37 b     |
| Ca²⁺ (meq kg⁻¹)               | 7.15±11.0 a    | 619±12.5 b      |

Plant uptake and translocation of DCD and guanidine under sterile conditions

DCD and guanidine uptake rates were determined under sterile conditions to determine if they were taken up intact (i.e., without prior microbial cleavage). Wheat seeds (Triticum aestivum var. Granary) were surface sterilised with 14% (v/v) NaClO and 80% ethanol and grown aseptically according to Hill et al. (2011). Briefly, surface sterilised seeds were germinated on agar containing 50% Murishage and Skoog basal medium, to screen for microbial contamination; after which they were transferred aseptically to Phytatrays (Sigma Aldrich, Gillingham, UK) containing sterile perlite and 50% Murishage and Skoog basal medium, supplemented with 10 mg l⁻¹ Na-metasilicate. Wheat plants were grown at 20 °C, with a 16 h photoperiod and light intensity (PAR) of 500 μmol m⁻² s⁻¹, until they had reached the third leaf stage. The roots of individual intact wheat plants (n=4) were placed in 12 ml of sterile (0.2 μm-filtered) solution containing either ¹⁴C-DCD or ¹⁴C-guanidine (ca. 1 kBq ml⁻¹; American Radiolabeled Chemicals, St Louis, MO, USA), for a period of 2 h. A solution concentration of 1 mM DCD was chosen to reflect the DCD concentration found within soil solution in response to typical field application rates (10 kg ha⁻¹) and two lower concentrations of 0.01 and 0.1 mM, were chosen to represent subsequent dilution of field applied DCD by diffusive and mass flow processes. Subsequently, the plant roots were thoroughly rinsed in 0.01 M CaCl₂, followed by deionised water and the roots and shoots oven dried (80 °C, 24 h). To quantify the ¹⁴C content of the plants, the roots and shoots were separately combusted in an OX400 biological oxidizer (RJ Harvey, Hillsdale, NJ, USA), the ¹⁴CO₂ captured in Oxosol scintillant (National Diagnostics, Atlanta, GA, USA) and ¹⁴C measured using a Wallac 1404 Liquid Scintillation Counter (Wallac EG&G, Milton Keynes, UK). To visualise the location of ¹⁴C-DCD and ¹⁴C-guanidine in the root and shoot tissues, the ¹⁴C distribution within intact plants was imaged using a Cyclone Plus phosphor-imaging system (PerkinElmer, Waltham, MA, USA) using an exposure time of 1 h.
Plant metabolism of DCD and guanidine

To determine whether DCD or guanidine could be mineralized within the plant, sterile wheat seeds were prepared as described previously. The roots of intact plants were then placed in sterile (0.2 μm-filtered) solutions containing either 14C-DCD or 14C-guanidine (4 ml; 1 kBq ml⁻¹; 0.01 mM). The plants were then placed in sterile 250 cm³ polypropylene vessels through which moist air was passed at a rate of ca. 600 ml min⁻¹. The outflow was bubbled through Oxosol scintillant to capture any respired ¹⁴CO₂. The Oxosol was changed after 1, 5, 10, 20, 40, 60, 90 and 120 min and captured ¹⁴C measured as described above.

Plant uptake of DCD and guanidine from soil

To determine the uptake rates of DCD and guanidine from soil, wheat seeds (n=4) were individually sown into Eutric Cambisol or Sapric Histosol rhizosphere microcosms (240 mm long; internal diameter 8 mm) as described in Owen and Jones (2001). Each microcosm contained approximately 12 g FW soil. Plants were grown under the same conditions used for the sterile uptake study (Section 2.2), until they had reached the third leaf stage. At this point, solutions of ¹⁴C-DCD or ¹⁴C-guanidine (ca. 1 kBq ml⁻¹; 0.01, 0.1 and 1 mM) were injected directly into the rhizosphere soil. A total of 4 injections (0.25 ml each) using 1 ml polypropylene syringes and 18 gauge needles at four depths (3, 9, 15 and 21 cm) were made, to facilitate an even distribution of solution within the microcosms. After 2 h, plants were removed from the microcosms and washed thoroughly in 0.01 mM CaCl₂, followed by distilled water. After drying (80 °C, 24 h), the ¹⁴C content of the root and shoot material was determined as described above.

To estimate the quantity of root in contact with the injected solution, blue ink was injected as above into another set of microcosms (n=4). The sections of root exposed to the ink were removed, washed, dried (80 °C) and weighed.

This experiment was repeated under similar conditions, utilising unlabelled DCD (1 mM), in order to establish whether the intact DCD molecule could be detected in wheat shoot extracts via HPLC. After injection of the substrate and washing of the root material, the shoot was separated from the root. The shoots of four wheat plants were bulked (n=3) in order to increase the likelihood of ascertaining a measurable peak on the HPLC, and ground in 2 ml of DMSO in a borosilicate Griffiths tube; 1 ml of the DMSO extract was then evaporated to dryness under vacuum in a rotary evaporator. The pellet was resuspended in 0.5 ml of HPLC grade water, centrifuged (10 000 g), and analysed for DCD using an adapted method of Turowski and Deshmukh (2004) on a Varian ProStar HPLC, with UV detection at 215 nm. The column used was a Luna 5u SCX (250×4.6 mm; 5 μm; 100 Å), DCD eluted after ca. four minutes in this system. Chromatograms were compared to standards and the control plants (no injected DCD).

DCD and guanidine mineralization within soils

DCD and guanidine mineralization were determined, over the same time course as the rhizosphere uptake study (Section 2.5), by measuring the rate of ¹⁴CO₂ evolution after the addition of 0.2 ml of ¹⁴C-DCD or ¹⁴C-guanidine (ca. 5 kBq ml⁻¹; 0.01, 0.1 and 1 mM) to 1 cm³ of each soil (n=4). Soils were contained in a 10 cm³ sealed glass vessel, with moist air flowing (ca. 100 ml min⁻¹) over the soil surface. Evolved ¹⁴CO₂ was captured by passing the outflow through two consecutive 0.1 M NaOH traps (capture efficiency >95 %; Hill et al. 2007). Traps were changed after 1, 5, 10, 20, 40, 60, 90 and 120 min, and the activity in the solution determined by liquid scintillation counting after mixing with HiSafe 3 scintillant (PerkinElmer, Llantrisant, UK).

DCD and guanidine microbial uptake and sorption within soils

The amount of substrate remaining in soil solution (i.e., that remaining after microbial uptake and abiotic removal processes) was determined according to the centrifugal-drainage procedure of Hill et al. (2008). Briefly, ¹⁴C-DCD or ¹⁴C-guanidine (0.2 ml; ca. 5 kBq ml⁻¹; 0.01, 0.1 or 1 mM) was pipetted evenly onto the soil surface. After 1, 5, 10, 20, 40, 60, 90 and 120 min, soil solution was recovered by centrifugation (4000 g, 1 min, 20 °C) and the amount of ¹⁴C-DCD or ¹⁴C-guanidine in the recovered soil solution determined by liquid scintillation counting as described above.

The amount of DCD or guanidine sorbed to the solid phase and present in soil solution was determined by performing 0.5 M K₂SO₄ extracts over time. Briefly, ¹⁴C-DCD or ¹⁴C-guanidine was mixed with soil and...
Results

DCD and Guanidine uptake and tissue localization in sterile wheat plants

Rates of uptake of 14C-DCD and 14C-guanidine by wheat roots increased ($p<0.001$) with increasing concentration (from 0.01 to 1 mM) in sterile solution (Table 2; Fig. 2). Phosphorimaging revealed a fairly

| Experimental condition | 14C-DCD | 14C-Guanidine |
|------------------------|--------|--------------|
|                        | 0.01 mM | 0.1 mM       | 1 mM          | 0.01 mM | 0.1 mM       | 1 mM          |
| Uptake rate (μmol g⁻¹ root DW h⁻¹) Sterile | 0.23±0.04 | 2.25±0.35 | 34.0±6.29 | 0.60±0.13 | 2.51±0.32 | 14.3±4.72 |
| Uptake rate (nmol g⁻¹ root DW h⁻¹) Eutric Cambisol | 3.02±0.92 | 30.9±6.17 | 353±94.3 | 7.13±1.88 | 49.8±13.9 | 328±66.0 |
| Uptake rate (nmol g⁻¹ root DW h⁻¹) Sapric Histosol | 2.18±0.57 | 21.1±1.72 | 174±13.4 | 5.25±0.77 | 47.2±14.7 | 211±39.2 |
| Shoot translocation (% of acquired label) Sterile | 7.46±1.18 | 7.76±0.50 | 15.0±4.05 | 22.4±3.96 | 19.0±6.58 | 22.0±3.18 |
| Shoot translocation (% of acquired label) Eutric Cambisol | 9.13±2.28 | 5.42±0.51 | 7.37±2.36 | 0.71±0.25 | 1.10±0.40 | 2.09±0.48 |
| Shoot translocation (% of acquired label) Sapric Histosol | 7.80±1.72 | 7.55±1.48 | 7.16±1.54 | 0.94±0.13 | 1.10±0.64 | 2.24±0.20 |
even distribution of the $^{14}$C label added as DCD within the shoot biomass (Table 2; Fig. 3), with the majority remaining in the roots. Phosphorimaging of $^{14}$C added as guanidine in the wheat plant showed an even distribution throughout the root system, but it was present predominantly in the lower regions of the shoot (Table 2; Fig. 3). Wheat roots acquired $2.37 \%$ less ($p<0.01$) of the $^{14}$C label added as DCD in comparison to guanidine at a concentration of 0.01 mM. No differences ($p>0.05$) were observed at 0.1 mM, while $1.71 \%$ more ($p<0.01$) DCD was acquired in comparison to guanidine at 1 mM.

Plant mineralization of assimilated DCD and guanidine

Of the added $^{14}$C-DCD and $^{14}$C-guanidine taken up by the plant, only small amounts were mineralized to $^{14}$CO$_2$ during the experiment. After 2 h, $0.44\pm0.07 \%$ of the acquired $^{14}$C-DCD and $0.90\pm0.22 \%$ of acquired $^{14}$C-guanidine had been metabolised to $^{14}$CO$_2$ within the plant with no differences observed between the two substrates ($p>0.05$). For both compounds, respiration rates tended to be faster in the first 20 min of the incubation (Fig. 4).

The top images represents the distribution of $^{14}$C-label within the plant tissue while, the images underneath are the corresponding photographs of the same plants.
DCD and guanidine uptake from soil rhizosphere microcosms

Wheat plants took up DCD-\textsuperscript{14}C and guanidine-\textsuperscript{14}C when grown in soil-filled microcosms (Fig. 5; Table 2), however, the rates were lower than those grown under sterile conditions. To evaluate the quantity of roots which were exposed to the injected \textsuperscript{14}C labelled substrates a blue ink tracer was injected into the microcosms. From this, we estimated that 41±8 and 32±5 % of the total root biomass was exposed to the injected \textsuperscript{14}C-substrates in the Eutric Cambisol and Sapric Histosol soil, respectively.

Increasing the concentration of injected \textsuperscript{14}C-DCD into soil rhizosphere microcosms increased (p<0.001) rates of root uptake of the label in both soil types (Table 2). Although a consistently higher mean root uptake rate of DCD was observed from the Eutric Cambisol in comparison to the Sapric Histosol at each concentration, differences were not significant (p>0.05). After 2 h, wheat roots had acquired 0.88±0.12, 0.96±0.12 and 1.09±0.21 % of total applied DCD-\textsuperscript{14}C (0.01, 0.1 and 1 mM respectively) from the Eutric Cambisol and translocated 5.42–9.13 % to the shoot material (Table 2); the amount acquired by wheat from the Sapric Histosol was ca. half this: 0.50±0.08, 0.53±0.06 and 0.44±0.06 % (0.01, 0.1 and 1 mM, respectively), with 7.16–7.80 % translocated to the shoot (Table 2).

Increasing the concentration of injected \textsuperscript{14}C-guanidine into the soil-filled microcosms increased rates of root uptake of guanidine-\textsuperscript{14}C in both soil types (p<0.001; Fig. 5). In the Eutric Cambisol, wheat roots acquired less (p<0.01) of the \textsuperscript{14}C label added as DCD in comparison to guanidine at 0.01 mM, yet uptake rates were similar (p>0.05) at 0.1 and 1 mM. In the Sapric Histosol, wheat roots acquired less (p<0.05) of the \textsuperscript{14}C label added as DCD in comparison to guanidine at 0.01

![Fig. 4 Cumulative mineralization of \textsuperscript{14}C-dicyandiamide (DCD) or \textsuperscript{14}C-guanidine in sterile wheat plants. Plants were exogenously supplied with each substrate (0.01 mM) for the whole 2 h monitoring period. Values represent mean±SEM (n=4)](image)

![Fig. 5 Uptake of \textsuperscript{14}C-dicyandiamide (DCD) or \textsuperscript{14}C-guanidine (0.01, 0.1 and 1 mM) by wheat plants grown in either a Eutric Cambisol (Panel A) or Sapric Histosol (Panel B), legend applies to both panels. Values represent means±SEM (n=4) and different letters indicate significant differences between means of different compounds, concentrations and between each soil type (Fisher’s LSD; p<0.05)](image)
and 1 mM, however, uptake rates were similar at 1 mM ($p>0.05$; Table 2). After 2 h, wheat roots had acquired $2.21\pm0.38$, $1.54\pm0.33$ and $1.00\pm0.09\%$ of total applied guanidine-$^{14}$C (0.01, 0.1 and 1 mM respectively) from the Eutric Cambisol and translocated $0.71\text{--}2.09\%$ to the shoot material (Table 2); the total amount acquired by wheat from the Sapric Histosol was $1.26\pm0.03$, $1.05\pm0.14$ and $0.50\pm0.08\%$ (0.01, 0.1 and 1 mM, respectively), with $0.94\text{--}2.24\%$ translocated to the shoot (Table 2). The percentage of acquired guanidine-$^{14}$C which was translocated to the shoot was lower than that of DCD-$^{14}$C in both soil types ($p<0.05$; Table 2).

Unlabelled DCD was detected within the wheat shoot extracts by HPLC, confirming intact uptake and translocation of the molecule to shoot material. The concentrations of DCD recovered in shoots following injection of 1 ml of 1 mM DCD and a 2 h incubation period were $79.7\pm19.2$ and $43.7\pm3.75\;\text{nmol DCD g}^{-1}\text{ shoot DW}$ in the Eutric Cambisol and Sapric Histosol, respectively. DCD concentrations in wheat shoots calculated from the $^{14}$C data showed the same higher shoot recovery in the mineral soil and were of the same order at $25.8\pm4.71$ and $9.48\pm2.31\;\text{nmol DCD g}^{-1}\text{ shoot DW}$ in the Eutric Cambisol and Sapric Histosol, respectively. Some disparity in the absolute values may have resulted from the different groups of plants used for the two methods of measurement.

Microbial mineralization of DCD and guanidine within soil

Over 2 h, the amount of $^{14}$C-DCD and $^{14}$C-guanidine mineralized in soil was small (Fig. 6; Table 3). After 2 h in the Eutric Cambisol, less ($p<0.01$) $^{14}$C-DCD was mineralized in comparison to $^{14}$C-guanidine, at 0.01 and 0.1 mM. Less $^{14}$C-DCD was also mineralized at 1 mM, however, differences were not significant ($p>0.05$). In the Sapric Histosol, more ($p<0.01$) $^{14}$C-guanidine was mineralized in comparison to $^{14}$C-DCD at the lowest concentration (0.01 mM); at 0.1 and 1 mM less ($p<0.05$) $^{14}$C-guanidine was mineralized in comparison to $^{14}$C-DCD. After 2 h, greater amounts of $^{14}$C-DCD were degraded in the Sapric Histosol in comparison to the Eutric Cambisol at all concentrations ($p<0.05$); conversely, greater amounts of $^{14}$C-guanidine were degraded in the Eutric Cambisol as opposed to the Sapric Histosol at all studied concentrations ($p<0.05$).
Microbial uptake and sorption of DCD and guanidine within soil

The amount of $^{14}$C-substrate added as DCD or guanidine present in the soil solution pool and the $\text{K}_2\text{SO}_4$-extractable pool is shown in Fig. 7. As the trends in the data were similar across all concentrations, only the 0.01 mM data are presented. Increasing applied DCD or guanidine concentration from 0.01 to 1 mM increased ($p<0.001$) the amount of DCD taken up by microbes in both soil types, at all concentrations (Table 3). Greater amounts of DCD and guanidine were taken up by microbes in the Sapric Histosol in comparison to the Eutric Cambisol at all studied concentrations ($p<0.001$). In the Eutric Cambisol a greater amount of added guanidine was taken up by microbes in comparison to DCD at 0.01 mM ($p<0.001$), and less guanidine was taken up in comparison to DCD at 0.1 and 1 mM ($p<0.001$). In the Sapric Histosol no difference was observed in the amount of substrate taken up by microbes as DCD or

![Fig. 7](image)

**Fig. 7** Amount of added $^{14}$C label recovered in the soil solution pool and in the 0.5 M $\text{K}_2\text{SO}_4$-extractable pool from a) $^{14}$C-dicyandiamide (DCD; 0.01 mM) added to Eutric Cambisol b) $^{14}$C-DCD (0.01 mM) added to Sapric Histosol c) $^{14}$C-guanidine (0.01 mM) in Eutric Cambisol and d) $^{14}$C-guanidine (0.01 mM) in Sapric Histosol. Legend applies to all panels and symbols represent means±SEM (n=4)

**Table 3** Summary of results for microbial mineralization, microbial uptake and sorption of $^{14}$C-DCD and $^{14}$C-guanidine (0.01, 0.1 and 1 mM) in a Eutric Cambisol and a Sapric Histosol after 2 h. Values represent means±SEM (n=4)

| Soil Type   | $^{14}$C-DCD |          |          | $^{14}$C-Guanidine |          |          |
|-------------|--------------|----------|----------|-------------------|----------|----------|
|             | 0.01 mM      | 0.1 mM   | 1 mM     | 0.01 mM           | 0.1 mM   | 1 mM     |
| Mineralization (% of applied label) | Eutric Cambisol | $1.58\pm0.08$ | $1.09\pm0.08$ | $0.89\pm0.37$ | $6.50\pm0.45$ | $2.13\pm0.24$ | $1.46\pm0.21$ |
| Mineralization (% of applied label) | Sapric Histosol | $2.82\pm0.21$ | $1.91\pm0.08$ | $2.40\pm0.31$ | $4.02\pm0.63$ | $1.12\pm0.23$ | $0.64\pm0.04$ |
| Microbial uptake (nmol g$^{-1}$ soil DW) | Eutric Cambisol | $0.19\pm0.004$ | $1.68\pm0.14$ | $15.2\pm0.75$ | $0.24\pm0.02$ | $1.48\pm0.09$ | $10.8\pm0.98$ |
| Microbial uptake (nmol g$^{-1}$ soil DW) | Sapric Histosol | $0.63\pm0.01$ | $5.97\pm0.18$ | $60.6\pm1.64$ | $0.68\pm0.01$ | $5.09\pm0.14$ | $50.1\pm1.36$ |
| Sorption (nmol g$^{-1}$ soil DW) | Eutric Cambisol | $0.28\pm0.002$ | $3.03\pm0.14$ | $32.0\pm0.57$ | $0.28\pm0.02$ | $3.78\pm0.07$ | $41.3\pm0.04$ |
| Sorption (nmol g$^{-1}$ soil DW) | Sapric Histosol | $0.60\pm0.01$ | $6.31\pm0.16$ | $62.2\pm1.68$ | $0.56\pm0.01$ | $7.33\pm0.12$ | $74.1\pm1.06$ |
guanidine at 0.01 mM ($p>0.05$) and a lower amount of guanidine was taken up by microbes at 0.1 and 1 mM ($p<0.01$), in comparison to DCD (Table 3).

Increasing either DCD or guanidine concentration from 0.01 to 1 mM increased the amount of substrate sorbed to both soil types at all studied concentrations ($p<0.001$; Table 3). A greater amount of either DCD or guanidine sorbed to the Sapric Histosol in comparison to the Eutric Cambisol at all studied concentrations ($p<0.001$). No difference was found in the amount of DCD or guanidine sorbed in either soil type at 0.01 mM ($p>0.05$), but greater amounts of guanidine sorbed in comparison to DCD in both soil types at concentrations of 0.1 and 1 mM ($p<0.001$; Table 3).

Discussion

Plant uptake

Our results clearly demonstrate that in the absence of competing physical and biological soil processes (e.g., sorption, microbial uptake and microbial degradation), wheat roots can acquire DCD and guanidine from solution. Interestingly, rates of uptake (34.0±6.29 µmol DCD g$^{-1}$ root DW h$^{-1}$; 14.3±4.72 µmol guanidine g$^{-1}$ root DW h$^{-1}$) at 1 mM under similar experimental conditions, were of a similar magnitude to other small N and C containing molecules found in soil (e.g., ca. 25 µmol NO$_3^-$ g$^{-1}$ root DW h$^{-1}$; ca. 23 µmol alanine g$^{-1}$ root DW h$^{-1}$; Hill et al. 2013); however, rates of uptake were greater for DCD and guanidine when considering moles of N acquired (136 µmol DCD-N g$^{-1}$ root DW h$^{-1}$ and 42.9 µmol guanidine-N g$^{-1}$ root DW h$^{-1}$ compared to 25 µmol NO$_3^-$-N g$^{-1}$ root DW h$^{-1}$ and 23 µmol alanine-N g$^{-1}$ root DW h$^{-1}$; Hill et al. 2013).

The mechanisms of DCD uptake and subsequent translocation within plants remain unknown. Being a synthetic compound, we hypothesise that no DCD-specific membrane transporters exist, however, it is possible that uptake could be facilitated by transporter proteins for structurally similar, naturally occurring molecules. A recent study by Eggen and Lillo (2012), found that a pharmaceutical drug used for diabetes II, metformin, had a high bioconcentration factor within seeds of Brassica napus. Like DCD, the drug metformin is structurally similar to the naturally occurring molecule, guanidine. The proposed mechanism of metformin entry to plant cells was via organic cation transporters (OCT), which transport naturally occurring N-containing compounds across the cell membrane. Expression of OCT has been demonstrated within several Arabidopsis tissues, including root tissue (Lelandais-Brière et al. 2007; Küfner and Koch 2008; Eggen and Lillo 2012). Substrates for plant OCT have not been well characterised, however, guanidine has been identified as a substrate for mammalian OCT (Cova et al. 2002). Assuming that DCD is an analogue of guanidine and acquired by the same mechanism, we would expect similar rates of uptake for both compounds. However, under sterile conditions we observed lower uptake rates of DCD compared to guanidine at 0.01 mM, similar rates at 0.1 mM and higher rates in comparison to guanidine at 1 mM. This suggests that the transporter affinity differs for the two compounds, and may also suggest there are other alternative transport pathways which need to be further investigated.

Plant translocation and assimilation

Lower amounts of DCD-$^{14}$C were recovered in the shoot in comparison to guanidine-$^{14}$C. However, the DCD-$^{14}$C appeared to be more evenly distributed within the shoot tissue. The low rates of mineralization of DCD- and guanidine-$^{14}$C within the plant over the short incubation time employed here may suggest saturation of metabolic pathways and thus that plant capacity to use the C and N acquired in these molecules is low. Recovery of intact DCD in wheat shoots by HPLC perhaps supports this view. However, preferential accumulation of structurally similar metformin, in seeds of Brassica napus and the occurrence of other guanidine derivatives, including arginine, in seeds may suggest storage of DCD and guanidine i.e., a lack of metabolic pathways which are directly connected to respiration (Ngamga et al. 2007; Eggen and Lillo 2012).

Competition for DCD and guanidine in the rhizosphere

Wheat was able to acquire ca. 0.5–1.0 % of the $^{14}$C applied as DCD and ca. 0.5–2 % of that applied as guanidine from soil within 2 h when in competition with the rhizosphere microbial community and sorption processes. Although realistic DCD soil solution
concentrations were chosen for this study, direct injection into the rhizosphere may have resulted in a greater amount of root surface area exposed to NI-containing soil solution than may be expected under field conditions. Actual DCD concentrations in the rhizosphere may vary according to NI application method, weather conditions, crop type, soil temperature and moisture and time since application. As only the C within molecules was isotopically labelled and detected within plant tissue, the results of the \(^{14}\text{C}-\text{DCD}\) uptake study do not unequivocally demonstrate that DCD was taken up intact from soil without prior lysis by soil microbes, or whether it remained intact once inside the plant without further degradation or transformation of the DCD molecule. Detection of unlabelled DCD within wheat shoots via HPLC, however, shows that intact uptake of DCD by plants took place.

Our results suggest that soil type is a regulator of DCD and guanidine bioavailability, with wheat acquiring consistent numerically (although not statistically) greater amounts of DCD-\(^{14}\text{C}\) and guanidine-\(^{14}\text{C}\) from the mineral Eutric Cambisol in comparison to the organic Sapric Histosol. A combination of a greater microbial uptake and sorption in the Sapric Histosol similarly suggests a lower availability for plant acquisition when compared to the Eutric Cambisol. The Sapric Histosol has a greater cation exchange capacity and more soil organic matter (which has been identified as important source of DCD binding domains (Jacinthe and Pichtel 1992; Zhang et al. 2004)) in comparison to the Eutric Cambisol, which may have led to greater sorption in this soil. The Sapric Histosol also had a greater microbial biomass compared to the Eutric Cambisol on a soil weight for weight basis, which may have caused greater amounts of DCD and guanidine to be taken up by microbes in this soil.

In comparison to some other simple C substrates (e.g., amino acids, sugars) and the level of sorption to soil particles, the mineralization of DCD and guanidine by soil microbes was very slow (Hill et al. 2008; Wilkinson et al. 2014). This might be expected considering that neither soil has previously been exposed to the synthetic nitrification inhibitor, DCD. The dissimilar pattern of DCD and guanidine mineralization, particularly at low concentrations in the Eutric Cambisol supports the tenet that DCD is not rapidly extracellularly degraded to guanidine. The greater degradation of DCD in the Sapric Histosol in comparison to the Eutric Cambisol may be explained by the greater microbial biomass on a soil weight for weight basis, although reasons for the lower rates of guanidine mineralization in the organic soil compared to the mineral soil are unclear. The low rates of guanidine uptake and mineralization at higher guanidine application rates (\(\geq 0.1 \text{ mM}\)) may also suggest that although naturally found in the microbial community, the capacity to internally assimilate high amounts of guanidine limits its metabolic conversion to CO\(_2\). This supports the conclusions of Rajbanshi et al. (1992) in that the microbial community requires longer time periods than 2 h to adapt to utilising DCD in soil. However, we cannot exclude the possibility that both DCD and guanidine are metabolised largely by pathways which do not feed into respiration.

Our results support the suggestion of Kelliher et al. (2014) that plants may play a role in reducing the half-life of DCD within soils, and this may be more pronounced in mineral soils as opposed to organic soils where plant uptake rates were greater due to lower amounts being sorbed and taken up by microbes. Interestingly, they also suggest that plants are able to derive some additional N from soils where DCD has been applied, and that guanidine (and perhaps other similar N-rich molecules) can be added to the growing list of naturally occurring N forms which plants are able to acquire from soil and metabolise. However, we hesitate to suggest that this has a significant role in plant N nutrition.

**DCD entry into the food chain**

There are three obvious routes of entry for DCD into meat or milk products: by i) consumption of pasture which has intercepted DCD on the foliage during spray application of the NI ii) direct livestock consumption of pasture or forage (which has acquired DCD from the soil as demonstrated here) and iii) ingestion of soil particles containing DCD.

Based on a dairy cow consuming 15 kg DM day\(^{-1}\) of grass (McDonald et al. 1996), with a standing biomass of 2000 kg DM ha\(^{-1}\) (O’Donovan and Dillon 1999), following a spray application of DCD (10 kg ha\(^{-1}\)), with 5 % of the total applied DCD intercepted via the canopy (Kim et al. 2012) we estimate that 3.5 g of DCD cow\(^{-1}\) day\(^{-1}\) could be consumed if allowed to graze immediately following application. Assuming that wheat is representative of other grasses and extrapolation of uptake and respiration rates to 24 h (with 50 %
translocation to shoots over this period), we estimate that 0.43 mg DCD cow⁻¹ day⁻¹ and 0.15 mg DCD cow⁻¹ day⁻¹ could be ingested following consumption of grass grown on the Eutric Cambisol and the Sapric Histosol, respectively. To estimate the amount of DCD consumed via soil ingestion we assume an even distribution of DCD in the top 10 cm of soil (soil bulk density of 1.10 and 0.31 g cm⁻³ for the Eutric Cambisol and Sapric Histosol, respectively) with removal due to plant uptake as above, and microbial mineralization at 1.30 and 5.93 % (Eutric Cambisol and Sapric Histosol, respectively) of applied DCD over 24 h (measured over this period by Scowen, M; unpublished). Based on 2 % of the cow’s DM intake being soil (Thornton and Abrahams 1983), 2.68 and 9.1 mg DCD cow⁻¹ day⁻¹ could be ingested with soil in the Eutric Cambisol and Sapric Histosol, respectively. Our calculations are based on a number of assumptions and upscaling laboratory data to field conditions, therefore, care should be taken when considering these estimations. Further research is required to elucidate how application methods (e.g., liquid vs. granular formulations) and environmental conditions (e.g., movement of NI into the root zone due to irrigation or rainfall) may influence pasture plant acquisition of this NI under field conditions.

Based on our estimates the magnitude of risk for DCD entry into the food chain via cattle follows the trend: pasture interception of DCD > soil ingestion of DCD > plant acquisition of DCD. However, the greatest risk pathway (canopy interception) would be transient (e.g., under high rainfall) and easily controlled by preventing grazing immediately following DCD application to pasture. Controlling the amount of DCD ingested beyond this point represents more of a challenge for out-grazing livestock and plant uptake may be a more significant pathway over longer periods. The potential for DCD to enter raw foods destined for direct human consumption (e.g., salad vegetables) also requires further investigation as the concentrations of DCD may be higher than in dairy products. Further, if like some other guanidine derivatives DCD accumulates in seeds, application of DCD to arable grain, oilseed or pulse crops could result in further direct DCD entry into the food chain due to the capacity of plants to acquire DCD from soil through roots (Ngamga et al. 2007; Eggen and Lillo 2012).

Acknowledgments This work was supported by the UK Natural Environment Research Council under grant award NE/IO12303/1. Partial funding for the research was also provided by an EU Knowledge Economy Skills Scholarship.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

Abalos D, Jefferey S, Sanz-Cobena A, Guardia G, Vallejo A (2014) Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. Agr Ecosyst Environ 189:136–144

Amberger A (1986) Potentials of nitrification inhibitors in modern N-fertilizer management. J Plant Nutr Soil Sci 149:469–484

Ball DF (1964) Loss-on-ignition estimate of organic matter and organic carbon in calcareous soils. J Soil Sci 15:84–92

Barneze AS, Minet AP, Cerrti CC, Misselbrook T (2015) The effect of nitrification inhibitors on nitrous oxide emissions from cattle urine depositions to grassland under summer conditions in the UK. Chemosphere 119:122–129

Bollard EG (1966) A comparative study of the ability of organic nitrogenous compounds to serve as sole sources of nitrogen for the growth of plants. Plant Soil 25:153–166

Chen X-H, Zhou L-X, Zhao Y-G, Pan S-D, Jin M-C (2014) Application of nanoring amino-functionalized magnetic polymer dispersive micro-solid-phase extraction and ultra fast liquid chromatography-tandem mass spectrometry in dicyandiamide residue analysis of powdered milk. Talanta 119:187–192

Cova E, Laforenza U, Gastaldi G, Sambuy Y, Tritto S, Faelli A, Ventura U (2002) Guanidine transport across the apical and basolateral membranes of human intestinal Caco-2 cells is mediated by two different mechanisms. J Nutr 132:1995–2003

Cui M, Sun X, Hu C, Di HJ, Tan Q, Zhao C (2011) Effective mitigation of nitrate leaching and nitrous oxide emissions in intensive vegetable production systems using a nitrification inhibitor, dicyandiamide. J Soils Sediments 11:722–730

Dai Y, Di HJ, Cameron KC, He J-Z (2013) Effects of nitrogen application rate and a nitrification inhibitor dicyandiamide on ammonia oxidizers and N₂O emissions in a grazed pasture soil. Sci Total Environ 465:125–135

Di H, Cameron KC (2004) Effects of temperature and application rate of a nitrification inhibitor, dicyandiamide (DCD), on nitrification rate and microbial biomass in a grazed pasture soil. Aust J Soil Res 42:927–932

Di H, Cameron KC (2006) Nitrous oxide emissions from two dairy pasture soils as affected by different rates of a fine particle suspension nitrification inhibitor, dicyandiamide. Biol Fertil Soils 42:472–480

Di H, Cameron KC, Shen JP, He JZ, Winefield CS (2009) A lysimeter study of nitrate leaching from grazed grassland as
affected by a nitrification inhibitor, dicyandiamide, and relationships with ammonia oxidizing bacteria and archaea. Soil Use Manag 25:454–461

Eggen T, Lillo C (2012) Antidiabetic II drug metformin in plants: uptake and translocation to edible parts of cereals, oily seeds, beans, tomato, squash, carrot and potatoes. J Agric Food Chem 60:6929–6935

Güthner T, Mertsenchek B, Schulz B (2014) Guanidine and Derivatives. In Wiley-VCH Ed. Ullmann’s Fine Chemicals, Wiley-VCH Verlag GmbH & Co. KGaA, Boschstr. 12, 69469, Weinheim, Germany

Hatch D, Trinidad H, Cardenas L, Carneiro J, Hawkins J, Scholefield D, Chadwick D (2005) Laboratory study of the effects of two nitrification inhibitors on greenhouse gas emissions from a slurry-treated arable soil: impact of diurnal temperature cycle. Biol Fertil Soils 41:225–232

Hill PW, Kuzyakov Y, Jones D, Farrar J (2007) Response of root respiration and root exudation to alterations in root C supply and demand in wheat. Plant Soil 291:131–141

Hill PW, Farrar JF, Jones DL (2008) Decoupling of microbial glucose uptake and mineralization in soil. Soil Biol Biochem 40:616–624

Hill PW, Quilliam RS, DeLuca TH, Farrar J, Farrell M, Roberts P, Newsham KK, Hopkins DW, Bardgett RD, Jones DL (2011) Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. PLoS ONE 6, e19220. doi:10.1371/journal.pone.0019220

Hill PW, Marsden KA, Jones DL (2013) How significant to plant N nutrition is the direct consumption of soil microbes by roots? New Phytol 199:948–955

Hutchinson HB, Miller NHJ (1912) The direct assimilation of inorganic and organic forms of nitrogen by higher plants. J Agric Sci 4:282–302

Jacinthe PA, Pichtel JR (1992) Interaction of nitrapyrin and dicyandiamide with soil humic compounds. Soil Sci Soc Am J 56:465–470

Jones DL, Willett VB (2006) Experimental evaluation methods to quantify dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biol Biochem 38:991–999

Kato T, Kondo T, Mizuno K (1986) Occurrence of guanidine compounds in several plants. Soil Sci Plant Nutr 32:487–491

Kawano M, Hwang J (2010) Influence of guanidine, imidazole, and some heterocyclic compounds on dissolution rates of amorphous silica. Clays Clay Miner 58:757–765

Kellihier FM, Clough TJ, Clark H, Rys G, Sedcole JR (2008) The temperature dependence of dicyandiamide (DCD) degradation in soils: a data synthesis. Soil Biol Biochem 40:1878–1882

Kellihier FM, van Koten C, Kear MJ, Sprosen MS, Ledgard FS, de Klein CAM, Letica SA, Luo J, Rys G (2014) Effect of temperature on dicyandiamide (DCD) longevity in pastoral soils under field conditions. Agric Ecosyst Environ 186:201–204

Kim D-G, Giltrap D, Saggar S, Palmenta T, Berpen P, Drysdale D (2012) Fate of the nitrification inhibitor dicyandiamide (DCD) sprayed on a grazed pasture: effect of rate and time of application. Soil Res 50:337–347

Kißner L, Koch W (2008) Stress regulated members of the plant organic cation transporter family are localized to the vacuolar membrane. BMC Res Notes, 1: doi: 10.1186/1756-0500-1-43

Ledgard SF, Menneer JC, Dexter MM, Kear MJ, Lindsey S, Peters JS, Pacheco D (2008) A novel concept to reduce nitrogen losses from grazed pastures by administering soil nitrogen process inhibitors to ruminant animals: a study with sheep. Agric Ecosyst Environ 125:148–158

Lelandais-Brière C, Jovanovic M, Torres GAM, Perry L, Corre-Menguy F, Hartmann C (2007) Disruption of AtOCT1, an organic cation transporter gene, affects root development and carmine-related responses in Arabidopsis. Plant J 51:154–164

Lewis AH (1936) The fertilizer value of some concentrated materials, particularly urea and guanidine and their nitrates and phosphates. J Agric Sci 26:509–526

Liu C, Wang K, Zheng X (2013) Effects of nitrification inhibitors (DCD and DMPP) on nitrous oxide emission, crop yield and nitrogen uptake in a wheat-maize cropping system. Biogeosciences Discuss 10:711–737

Macadam XMB, del Prado A, Merino P, Esatvillo JM, Pinto M, González-Murua C (2003) Dicyandiamide and 3, 4-dimethyl pyrazole phosphate decrease N₂O emissions from grasslands but dicyandiamide produces deleterious effects in clover. J Plant Physiol 160:1517–1523

McDonald P, Edwards R, Greenhalgh JFD (1996) Animal nutrition. Longman Group Limited, Essex

Minet EP, O’Carroll C, Rooney D, Breslin C, McCarthy CP, Gallagher L, Richards KG (2013) Slow delivery of a nitrification inhibitor (dicyandiamide) to soil using a biodegradable hydrogel of chitosan. Chemosphere 93:2854–2858

Ministry for Primary Industries (2013) [WWW document] URL http://www.mpi.govt.nz/Portals/0/Documents/news-resources/news/letter-of-assurance.pdf [accessed 1 September 2014]

Miranda KM, Epsey MG, Wink DA (2001) A rapid, simple, spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide-Biol Ch 5:62–71

Mulvaney RL (1996) Nitrogen - inorganic forms. In: Sparks DL (ed) Methods of soil analysis. Part 3. Soil Science Society of America Inc, Madison, pp 1123–1184

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27:31–36

Nganga D, F anso Free SNY, Tane P, Fomum ZT (2007) Millaurine A, a new guanidine alkaloid from seeds of Millettia laurentii. Fitoterapia 78:276–277

O’Callaghan M, Gerard EM, Carter PE, Lardner R, Sarathchandra U, Burch G, Ghani A, Bell N (2010) Effect of the nitrification inhibitor dicyandiamide (DCD) on microbial communities in a pasture soil amended with bovine urine. Soil Biol Biochem 42:1425–1436

O’Connor PJ, Hennessy D, Lynch MB, Sletteny H, Lewis E (2013) The effect of dicyandiamide on rumen and blood metabolites, diet digestibility and urinary excretion. Livest Sci 155:35–37

O’Donovan M O, Dillon P (1999) Measurement of grassland management practice on commercial dairy farms: TEAGAS C: Co. Cork, Ireland. 4351:1

Owen AG, Jones DL (2001) Competition for amino acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biol Biochem 33: 651–657

Prescott AG, John P (1996) Dioxygenases: molecular structure and role in plant metabolism. Annu Rev Plant Phys 47:245–271
Rajbanshi SS, Benckiser G, Ottow JCG (1992) Mineralization kinetics and utilization as an N source of dicyandiamide (DCD) in soil. Naturwissenschaften 79:26–27
Reddy GR (1964) Effect of varying quantities of dicyandiamide on the utilization of nitrogen by several crops from sodium nitrate and ammonium sulphate. J Agr Sci 62:35–38
Schulten HR, Schnitzer M (1998) The chemistry of soil organic nitrogen: a review. Biol Fert Soils 26:1–15
Subbarao GV, Sahrawat KL, Nakahara K, Ishikawa T, Kishii M, Rao IM, Hash CT, George TS, Rao PS, Nardi P, Bonnett D, Berry W, Suenaga K, Lata JC (2012) Chapter six - Biological nitrification inhibition - a novel strategy to regulate nitrification in agricultural systems. In: Sparks, D.L., eds. Advances in Agronomy, Academic Press 114: 249–302
Thornton I, Abrahams P (1983) Soil ingestion – a major pathway of heavy metals into livestock grazing contaminated land. Sci Total Environ 28:287–294
Turowski M, Deshmukh B (2004) Direct chromatographic method for determination of hydrogen cyanamide and dicyandiamide in aqueous solutions. Anal Lett 9:1981–1989
Voroney RP, Brookes PC, Beyaert RP (2008) Soil microbial biomass C, N, P and S. In: Carter MR, Gregorich EG (eds) Soil sampling and methods of analysis, 2nd edn. CRC Press, Boca Raton, pp 637–651
Warren CR (2014) Organic N molecules in the soil solution: what is known, what is unknown and the path forwards. Plant Soil 375:1–19
Weiske A, Benckiser G, Herbert T, Ottow J (2001) Influence of the nitrification inhibitor 3,4-dimethylpyrazole phosphate (DMPP) in comparison to dicyandiamide (DCD) on nitrous oxide emissions, carbon dioxide fluxes and methane oxidation during 3 years of repeated application in field experiments. Biol Fert Soils 34:109–117
Welten BG, Ledgard SF, Schipper LA, Judge AA (2013) Effect of amending cattle urine with dicyandiamide on soil nitrogen dynamics and leaching of urinary-nitrogen. Agr Ecosyst Environ 167:12–22
Wilkinson A, Hill PW, Farrar JF, Jones DL, Bardgett RD (2014) Rapid microbial uptake and mineralization of amino acid and peptide-N along a grassland productivity gradient. Soil Biol Biochem 72:75–73
Zerulla W, Barth T, Dressel J, Erhardt K, von Locquenghien KH, Pasda G, Rädle M, Wissemeier AH (2001) 3,4-Dimethylpyrazole phosphate (DMPP) – a new nitrification inhibitor for agriculture and horticulture. Biol Fert Soils 34:79–84
Zhang HJ, Wu ZJ, Zhou QX (2004) Dicyandiamide sorption–desorption behaviour on soils and peat humus. Pedosphere 14:395–399